THE AMINO ACID CONTENT AND NUTRITIVE VALUE OF THE PROTEINS OF COTTONSEED MEAL

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THE AMINO ACID CONTENT AND NUTRITIVE VALUE OF THE PROTEINS OF COTTONSEED MEAL

I. INTRODUCTION

A. CHEMISTRY OF THE PROTEINS OF COTTONSEED MEAL

NATURE OF THE PROTEINS. One of the earliest references to the composition of the proteins of cottonseed meal was made by Ritthausen (1) in 1881. He states that he repeatedly attempted to prepare crystalline proteins from cottonseed cake, peanuts, sunflower seed and other substances, but without success, altho he did separate the proteins in the form of spheroids.

Osborne and Voorhees (2) studied the proteins of cottonseed meal quite extensively. The protein obtained in the purest state had the nature of a globulin, being soluble in salt solutions, and comprised 15.83 per cent of the air dry and oil free meal and contained 42.3 per cent of the total nitrogen of the meal. Another protein (or proteins) was found to be insoluble in salt solutions but soluble in 0.2 per cent potash solution and amounted to 44.3 per cent of the total nitrogen of the meal. Two per cent of the total nitrogen was present in the form of water soluble proteose. Thus the remaining nitrogenous matter, containing 11.4 per cent of the total nitrogen, was insoluble in both salt and alkaline solutions. The globulin was given the name "Edestin" from the Greek word "Edestos", signifying edible, since it agreed in composition with and had properties similar to the protein prepared from the seeds of wheat, maize, hemp, castor bean, squash and flax.

The following table presents a comparison of the composition of the edestin of cottonseed with that of other seeds, the figures given for the former being the average of the results of the analyses of six of the purest preparations.

COMPOSITION OF EDESTIN FROM VARIOUS SEEDS

	Cotton seed	Wheat kernel	Maize kernel	Hemp seed	Castor bean	Squash seed	Flax seed
Carbon	51.71	51.03	51.71	51.28	51.31	51.66	51.48
Hydrogen	6.86	6.85	6.85	6.84	6.97	6.89	6.94
Nitrogen	18.64	18.39	18.12	18.84	18.75	18.51	18.60
Sulphur	0.62	0.69	0.86	0.87	0.76	0.88	0.81
Oxygen	22.17	23.04	22.46	22.17	22.21	22.06	22.17
	100.00	100.00	100.00	100.00	100.00	100.00	100.00

THE DISTRIBUTION OF THE NITROGEN IN THE INDIVIDUAL PROTEINS. The distribution of nitrogen in various protein bodies was studied exhaustively by Osborne and Harris (3), who employed the Hausmann method (4), modified in some particulars. The following table is typical of their results.

PERCENTAGE OF NITROGEN IN THE DIFFERENT GROUPS IN VARIOUS PROTEIN BODIES

Source	Nitrogen as ammonia	Basic nitrogen	Non-basic nitrogen	Nitrogen in MgO precipitate	Total nitrogen
Globulin, cotton seed	1.92	5.71	11.01	----	18.64
Globulin, wheat	1.42	6.83	9.82	0.28	18.39
Globulin, flax seed	2.00	4.77	11.49	0.22	18.48
Zein, maize	2.97	0.49	12.51	0.16	16.13
Hordein, barley	4.01	0.77	12.04	0.23	17.21

These investigators emphasize the fact that "The most striking feature shown by this table is the wide range in the amount of basic nitrogen obtained from the different proteins." They state further "This wide variation in the proportion of basic decomposition products of the various proteins, as Kossel and Kutscher point out, raises important questions regard-

ing their food value."

In a study of the forms of nitrogen precipitated by phosphotungstic acid, Osborne (5) determined the amounts of the three basic amino acids, histidine, arginine and lysine, in a number of proteins. It was assumed in making the calculations that no other amino acids than the three mentioned were precipitated by the phosphotungstic acid. Some of the results expressed in percentage of the protein, are presented below.

FORMS OF NITROGEN PRECIPITATED BY PHOSPHOTUNGSTIC ACID

	Histidine	Arginine	Lysine	Basic N calc.	Basic N ppt'd.	Difference	Basic N in p.c. of that precipitated
Globulin, cotton seed	3.46	13.51	2.06	5.69	5.71	-0.02	99.65
Legumelin, soy bean	2.04	5.35	4.91	3.21	3.08	0.13	104.22
Gliadin, wheat	0.58	3.16	0.00	1.18	1.09	0.09	108.30
Hordein, barley	1.28	2.16	0.00	1.05	0.77	0.28	136.40
Zein, maize	0.82	1.35	0.00	0.65	0.49	0.16	132.60

The content of the mono-amino acids of the "edestin" of cottonseed meal was determined by Abderhalden and Rostoski (6) by the use of the Fischer ester method (7), and is as follows, calculated for dry, ash free edestin of cottonseed:-

Glycocoll	1.2 per cent
Alanin	4.5 " "
Amino valerianic acid	present
a-proline	2.3 per cent
Leucine	15.5 " "
Glutamic acid	17.2 " "

Aspartic acid	2.9 per cent
Phenyl alanin	3.9 " "
Serin	0.4 " "
Tyrosin	2.3 ". "
Tryptophane	present

These results are stated to agree very closely in the proportionate quantity of amino acids to the edestin of hemp seed.

THE DISTRIBUTION OF NITROGEN IN THE COMBINED PROTEINS. The quantitative determination of the amino acids of feedingstuffs by means of the Van Slyke method (8) was undertaken by Grindley and his co-workers (9). In this investigation, the methods of Van Slyke were followed to a considerable extent, but larger quantities of the original material were used than in the case of the protein substances analyzed by Van Slyke. The experimenters state that the results "indicate that the Van Slyke method for the determination of the chemical groups characteristic of the amino acids can be applied directly to the quantitative determination of the amino acids of feedingstuffs with at least a fair degree of accuracy." The following table includes some of the data obtained, expressed in per cent of the total nitrogen of the feedingstuff.

THE QUANTITATIVE DETERMINATION OF THE AMINO ACIDS OF FEEDINGSTUFFS

Feedingstuffs	Ammonia N	Melanine N	Arginine N	Cystine N	Histidine N	Lysine N	Amino N in filtr. from bases	Non-amino N in filtr. from bases	Total N by summation
Cotton-seed meal	10.45	8,78	19.52	0.65	5.47	4.78	42.82	5.43	96.90
Tankage	6.58	4.40	14.15	1.28	4.97	7.48	52.39	7.27	98.49
Alfalfa hay	8.44	15.79	7.68	0.88	7.44	4.10	44.02	9.79	98.08

About 1915 Nollau (10) attempted to apply the Van Slyke (8) method of analysis to a study of the distribution of the nitrogen in the proteins of feedingstuffs. Samples of the finely ground feeds were hydrolyzed with twenty per cent hydrochloric acid until the content of amino acid, as determined by the Van Slyke method, became constant. The material insoluble in hydrochloric acid was filtered off, the clear extract concentrated under diminished pressure and made up to a certain volume. The total nitrogen content of this extract was used as a basis for calculating the final results. Some of the data obtained are shown in the table below.

DISTRIBUTION OF NITROGEN IN VARIOUS PROTEIN SUBSTANCES

Protein substance	Ammonia N	Melanine N	Cystine N	Arginine N	Histidine N	Lysine N	Mono amino acid N (Amino N of filtrate)	Prolin, oxyprol, tryp. etc. (Non-amino N of filtrate)	Total
Cottonseed meal	14.06	6.27	2.74	12.77	7.57	1.94	45.02	7.49	97.86
Maize kernel	4.63	7.00	4.06	16.19	4.45	8.53	46.69	0.00	94.55
Oat grain	13.31	2.97	4.48	11.42	9.58	0.00	43.49	11.29	96.54
Barley grain	16.19	2.87	4.38	8.65	6.70	0.00	44.16	18.37	101.32
Rye grain	15.00	1.54	2.20	10.49	10.48	1.24	37.96	21.36	100.52
Gluten (wheat)	22.53	1.01	1.91	7.61	5.57	0.51	49.05	9.76	97.95

A report of the subsequent work of Grindley and his co-workers (11) shows that the results obtained by them do not agree in detail with those obtained by Nollau. It is claimed by the former that since Nollau filtered off the solid residue after hydrolysis of the feedingstuff and before making his total nitrogen determinations upon which the final calculations were based, that his results cannot be used to calculate the amino acid content

of the feeds since a part of the total nitrogen was undoubtedly discarded in the solid residue. The sulphur determinations of Nollau are from two to four times as high as those of Grindley, et al., and hence his lysine values are much lower than those of the latter investigators.

Of the feedingstuffs cottonseed meal, alfalfa hay, barley, whole wheat, rolled wheat, white soy beans, oats, tankage and blood meal, analyses of which were made by Grindley and his co-workers, the first was found to have the lowest content of mono-amino acid nitrogen, 48.25 per cent, and correspondingly the highest diamino acid nitrogen content, 30.42 per cent, (expressed in per cent of the total nitrogen of the feed). Likewise cottonseed meal was found to have the highest arginine content of any of the feeds mentioned, 19.52 per cent, and the lowest cystine content, 0.65 per cent, while in histidine and lysine content cottonseed meal occupies a medium position.

HEATS OF COMBUSTION OF THE PROTEINS. The heats of combustion of several vegetable proteins were carefully determined by Benedict and Osborne (12). The following table includes a few of the results of their work. The results, expressed in percentage, are calculated for one gram of dry substance.

HEATS OF COMBUSTION OF VARIOUS PROTEINS

	C.	H.	N.	S.	O.	Calories per gram
Globulin, cotton seed	51.71	6.86	18.30	0.62	22.51	5596
Glycinin, soy bean	52.01	6.89	17.47	0.71	22.92	5668
Gliadin, wheat, rye	52.72	6.86	17.66	1.03	21.73	5738
Globulin, wheat	51,03	6.85	18.30	0.69	23.13	5358
Hordein, barley	54.29	6.80	17.20	0.85	20.86	5916

In commenting upon their determinations, the investigators state;

"In general the higher heats of combustion are found for those proteins which have a higher carbon content and similarly for those with lower oxygen content. Many irregularities, however, appear in the preceding table which are doubtless due to the different proportion of the various amino acids which constitute the molecules of the different proteins."

SUMMARY OF DISCUSSION OF CHEMISTRY OF THE PROTEINS. It is evident from the foregoing discussion that our knowledge of the composition of the proteins of cottonseed meal is very incomplete. The globulin is the only protein of the cottonseed which has been isolated in pure form and whose composition has been determined. The globulin, however, according to Osborne and Voorhees (2), contains only 42.3 per cent of the total nitrogen of the cottonseed. The character, identity and chemical composition of the remaining proteins are practically unknown, and it is evident from the data given above that our knowledge of the distribution of the nitrogen in the proteins of cottonseed meal is very meager indeed. The investigation of the distribution of the nitrogen in the proteins of cottonseed meal, is therefore, a field of study which should be undertaken from both the scientific and economic standpoints.

B. THE NUTRITIVE VALUE OF THE PROTEINS OF COTTONSEED MEAL

UTILIZATION. Fraps (13) gives the utilization of the proteins of cottonseed meal by steers or sheep as 88.4 per cent. Henry and Morrison (14) give the digestibility of the protein of choice and prime cottonseed meal as 84 per cent. Mendel and Fine (15), in a study of the utilization of the proteins of the cottonseed by dogs found that the proteins of cottonseed flour containing 7.4 per cent nitrogen were not assimilated to nearly so great an extent as the proteins of meat. The utilization of the nitrogen of cottonseed flour by dogs ranged from 67 to 75 per cent, while the nitrogen of meat

given in diets containing comparable or greater amounts of indigestible material, was utilized to the extent of 88 to 93 per cent.

Rather (16) compared the digestibility and utilization of the proteins of cottonseed meal and flour with the proteins of meat using men as subjects. He found that the utilization value for the proteins of cottonseed meal ranged from 74.7 to 80.2 per cent with an average of 77.6 per cent, while the proteins of cottonseed flour gave values of 79.2 to 79.7 per cent and averaged 79.5 per cent. No significance was attached to the small differences between cottonseed meal and cottonseed flour. Much higher figures were obtained for the proteins of meat, the average utilization being 96.6 per cent.

The coefficients of digestibility of the proteins of the cottonseed when the gastric juice of the dogsis employed, are reported by Pomaski (17) to be from 99 to 100 per cent.

NUTRITION EXPERIMENTS. Richardson and Green (18) have published a series of papers relating to the nutritional value of cottonseed meal and flour. Albino rats were employed as the experimental animals. Cottonseed meal and flour were found to be satisfactory sources of protein in the diet of rats. The proteins in diets containing 50 per cent cottonseed flour, protein free milk, and butterfat, were found to suffice for normal growth and development and for reproduction to the third generation. The addition of 5 per cent of casein to such a diet caused no better growth, but was inducive to more rapid reproduction. While a diet containing 18 per cent of cottonseed protein when supplemented with sufficient amounts of all other substances necessary for nutrition induced practically normal growth in the male rat and better than average in the female, such a diet caused high mortality in the litters of the second generation. Both the males and females of the second generation were slightly undersize at 148 days of age. With diets containing 12 per cent or less of cottonseed protein, normal growth was not se-

cured, but on diets containing as little as six per cent cottonseed protein, live weights were maintained for a considerable period.

Mendel (19) states that normal growth has been secured for considerable periods when the following proteins have been fed individually in suitable concentration; globulin of cottonseed; glutelin of maize; glutenin of wheat; glycinin of the soy beah; edestin of hemp seed.

In observations of the growth of albino rats during feeding investigations with isolated food substances, Osborne and Mendel (20) found that adequate growth was secured on a ration containing 18 per cent cottonseed globulin, the balance of the ration being composed of "protein free milk", starch and fat.

In studying the effect of the amino acid content of the ration on the growth of chickens, the same investigators (21) found that "in further accord with the observations of the growth of rats, cottonseed flour forms a suitable adjuvant for the proteins of corn gluten, whereby in the presence of "protein free milk", butterfat, etc., satisfactory increments of growth can be obtained."

In further studies of the relative value of certain proteins as supplements to corn gluten, Osborne and Mendel (22) prepared the proteins of commercial cottonseed flour by extracting the flour by sodium hydroxide solution and then precipitating the proteins by neutralization. The use in the diet of proteins prepared in this manner demonstrated the "pronounced efficiency" of the proteins of cottonseed as supplements to corn gluten, while the proteins of brewers' grains and "vegetable albumin" flour were shown to be comparatively inefficient. When one fifth to one fourth of the corn gluten protein was replaced by the protein of cottonseed flour, the rats were enabled to make more rapid growth than when one half or as much as two thirds of the totalnitrogen was replaced by the proteins of brewers'

grains or "vegetable albumin flour." The inefficiency of the corn gluten is attributed to its low content of lysine and tryptophane.

Osborne and Mendel (23), in an extensive investigation regarding the use of cottonseed as food found that in "feeding experiments on rats in which these proteins furnished practically all of the food nitrogen and in which the other essential dietary components were supplied by adding to the products to be tested a suitable mixture of "protein free milk", butter fat and starch which with the addition of adequate protein, has been shown in hundreds of experiments to be sufficient for perfect growth, ---- satisfactory growth can be made by rats when either cottonseed globulin or the total cottonseed protein precipitated from alkali extracts of cottonseed meal is employed without other significant protein sources in the mixture. In experiments in which the inorganic components were furnished by our "artificial protein free milk" there was no failure of growth when the cottonseed meal was used, thus suggesting that the latter contains the equivalent of the 'determinant', 'food accessory', or 'vitamin' deemed essential for nutrition and furnished in fat free milk." Since "satisfactory growth" was attained on diets containing an equivalent of 9 per cent of cottonseed protein and "considerable growth" was made on diets containing 6 per cent protein, these experimenters state that this attests the excellent quality of cottonseed proteins.

The high nutritive value of cottonseed proteins was further demonstrated in the above mentioned experiments by employing cottonseed flour as a supplement to "inferior" protein concentrates such as corn gluten, distillers' grains and "vegetable albumin flour." These protein concentrates are deficient or poorly balanced in their amino acid content, but with the use of cottonseed proteins as a supplement, good growth was secured.

McCollum and Simmonds (24), as a result of experiments designed to test

the value of some seed proteins for maintenance, conclude that "cottonseed proteins are of relatively good quality as indicated by the maintenance of body weight in all rats fed a ration whose 6 per cent protein content was derived from this source."

The relation of the quality of proteins to milk production has been studied in detail by Hart and Humphrey (25). In these investigations, in which dairy cows were employed, the concentrates furnished approximately 40 per cent of the digestible protein of the rations and were used to supplement a basal ration of corn meal, corn silage and clover hay. The total protein formed 12 per cent of the dry matter. While earlier experiments had shown the efficiency of the proteins of corn meal and corn stover, these experiments showed an equality in efficiency of the proteins of gluten feed, oil meal, distillers' grains and cottonseed meal as supplements to the protein of corn meal and clover hay. In a later series of experiments (26) of the same nature, the concentrates furnished approximately 37 per cent of the protein of the rations and were used to supplement a basal ration of corn silage and alfalfa hay. In these experiments, about ten per cent of the dry matter of the ration consisted of protein and the nutritive ratio was 1:8.4. The efficiency of the various concentrates employed was calculated on the basis of the total nitrogen absorbed by the animals. In the case of different experimental animals, the nitrogen of the milk plus or minus that of tissue formed or destroyed was found to comprise 28 per cent, 35 per cent, and 42 per cent, respectively, of the nitrogen absorbed from a ration containing cottonseed meal. When gluten feed was employed as the concentrate, the efficiency of the same animals was found to be 43 per cent, 40 per cent and 43 per cent, respectively. Oil meal showed an efficiency of 39 per cent, 40 per cent, and 47 per cent, while distillers' grains had the highest efficiency, 50 per cent, 51 per cent and 53 per cent, respectively.

SUMMARY OF DISCUSSION OF NUTRITIVE VALUE OF THE PROTEINS. From a review of the literature, it is apparent that investigations upon the nutritive value of the proteins of cottonseed meal are quite limited in extent. In the majority of experiments cited, the investigators draw their conclusions from the maintenance of live weight, increase in live weight, state of health or combinations of these creteria. In most cases the amount of food consumed is not recorded, so that it is impossible to judge whether or not the results secured were due to a failure of the animals to consume a sufficient amount of certain rations in order to cover their energy requirements. In but one series of experiments (25,26), were the conclusions based upon metabolism studies. In these studies, however, the nutritive value of the cottonseed meal proteins was not studied by the use of rations in which cottonseed meal formed the sole source of protein, but on the contrary these proteins formed only 37 per cent to 40 per cent of the total content of digestible protein furnished from three or more sources. It is not evident, therefore, whether the results obtained represent the true nutritive value of cottonseed meal proteins for milk production or whether their nutritive value was actually lowered due to the simultaneous interaction of the three or more kinds of protein in the ration. Hence, the further study of the nutritive value of the proteins of cottonseed meal is desirable.

TOXICITY OF COTTONSEED MEAL PROBABLY NOT DUE TO PROTEINS. The results of numerous feeding experiments, both in this country and in Europe indicate that "swine - particularly young pigs - calves, sheep, horses, cows, steers, dogs, cats, guinea pigs, rabbits, fish, poultry and other animals may be injured by eating cottonseed meal. Some of the smaller animals, such as pigs and calves, seem to be more susceptible to its injury than cows, steers and similar animals. This, however, may have been due to their youth, or, more probably, to a consumption of larger quantities of meal in proportion to

their live weight. When the meal was fed in connection with pasturage, or when it had been steamed, boiled or fermented, or when fed with mineral matter, particularly iron compounds, it often seemed to exert no apparent injury to pigs even when fed in rather large quantities.

"The injury resulting from the feeding of cottonseed meal to stock has been attributed to; (a) the oil in the meal; (b) its crude fiber; (c) excess of nitrogen and perverted metabolism; (d) the action of bacteria and molds; (e) presence of betain, cholin, or other alkaloids, and to gossypol; (f) to injurious phosphorus compounds; (g) to a protein group containing loosely bound sulphur, which interferes with normal iron metabolism; to worms and certain other causes, which, perhaps, are not of sufficient merit to warrant discussion here." (27)

From an examination of the literature, it would seem that there is but little basis for attributing the toxicity of cottonseed meal to its proteins. The assumption that the high protein content of cottonseed meal was responsible for its harmful effects (28), was examined by Dinwiddie (29), who maintains that this theory is "not supported by a study of the recorded feeding tests. In those conducted by the experiment stations cottonseed meal has been usually fed in combination with ground corn or kafir corn so as to form an approximately balanced ration. Moreover, our own experiments show a lesser fatality in the more highly nitrogenous ration, cottonseed meal and bran, than in the wider ration of cottonseed meal and corn."

Wells and Ewing (27), however, believe that "In a restricted ration, such as used in one series, pigs were seriously injured or killed within four to six weeks by eating digester tankage in amounts of nitrogen equivalent to that in a provisional lethal dose of cottonseed meal, which was approximately 15 grams daily for each pig. This would indicate that, if cottonseed meal is fed in a restricted ration and in large quantities , the ration may injure

and kill pigs, even though it should contain no specific toxic substance."

Withers and Brewster (30) were led as a result of their experiments to the hypothesis that the toxic principle of cottonseed meal is a certain group of the protein molecule which contains loosely bound sulphur, and that the injurious effects of the meal are due to the reaction between this group and the iron of the blood. Later work by Withers and associates (31), however, led them to conclude that the toxicity of cottonseed meal is due to the presence of "gossypol", a definite chemical compound soluble in ether and aniline. They believe "gossypol" may be changed to a nearly related substance "D-gossypol", the latter being insoluble in ether but soluble in aniline. When in alcoholic solution either of these compounds forms precipitates with the alcohol soluble proteins of wheat flour and of cottonseed meal. They reason that the reduction of the toxicity of cottonseed meal by heating may be due to the inability of the animal to digest the "gossypol", or "D-gossypol" protein compound.

In their series of feeding experiments with albino rats, Richardson and Green (18) observed no toxic effects. Rations containing forty to fifty per cent cottonseed flour were fed to rats thru four successive generations and to one individual for 565 days. Osborne and Mendel (23), also employing rats as experimental animals, state that "No toxic symptoms have appeared, even when the supposedly harmful meal also was used, during a period in which the animals attained a large size." On the other hand, in feeding experiments with the cottonseed kernels themselves it was found that a toxic substance is present which renders rations containing the kernels unsatisfactory for the nutrition of rats.

SUMMARY OF DISCUSSION OF TOXICITY. In the light of the foregoing discussion, it seems very doubtful if the toxicity of cottonseed meal may be attributed to either its high protein content or to the character of the

proteins which it contains. Further, it seems clear that commercial cottonseed meal or flour of good quality may provide practically the entire nitrogenous components of the ration for albino rats over a long period of time with no injurious effects becoming manifest.

II. METHODS EMPLOYED IN THE CHEMICAL ANALYSIS OF THE PROTEINS OF COTTONSEED MEAL[1]

PREPARATION OF SAMPLE

A sack of good quality commercial cottonseed meal was purchased on the market. The meal had a bright yellow color and was comparatively free from hulls. The meal was emptied on a clean concrete floor and thoroughly mixed. The amount was reduced by quartering until two quarters formed the amount desired for the sample to be analyzed. This sample was finely ground, passed through a forty mesh sieve and thoroughly mixed.

A. EXTRACTION WITH ABSOLUTE ETHER

Four portions of 15 grams each of the finely ground sample of cottonseed meal were weighed off and placed in 500 c.c. centrifuge bottles. Two hundred c.c. of absolute ether were added. The bottles were corked securely and the corks wired. The bottles were placed on a mechanical shaker and the meal allowed to digest. The time of the extraction during the day was 7 to 8 hours, and the time of the extraction over night 14 to 15 hours. After each extraction, the sides of the bottles were washed down with a few c.c of

Note[1]: The method of procedure here outlined is one which has been developed and perfected in this laboratory by Dr. H. S. Grindley, Mr. T. S. Hamilton and associates (9, 11, 32, 33 and unpublished manuscripts). The method of extraction preliminary to hydrolysis of the proteins has been developed entirely in this laboratory, while the actual determination of the nitrogen in the different groups follows closely the method of Van Slyke (8), but includes modifications perfected in this laboratory. The entire procedure is included here for the sake of completeness.

ether. The bottles were placed in the centrifuge and rotated until the solid matter formed a compact mass. The clear liquids were decanted into properly labeled flasks, This treatment was repeated six times using each time 100 c.c of absolute ether. Each of the combined ether extracts was filtered by gravity thru a Whatman filter No. 1, using a 4 inch funnel. The flasks and filters were washed with ether. The filtrates were made slightly acid with sulphuric acid and the ether recovered by distillation in Kjeldahl flasks on the steam bath. Total nitrogen determinations were made on each of the ether extract residues.

The solid matter collected on the filters was added to the residues insoluble in absolute ether and the latter treated as described below in (B).

B. EXTRACTION WITH COLD ABSOLUTE ALCOHOL

Two hundred c.c.of absolute alcohol were added to each of the bottles containing the residues insoluble in ether. The bottles were placed on the shaker as before. The time of the extraction during the day was 7 to 8 hours and the time of the extraction over night 14 to 15 hours. After each extraction the sides of each bottle were washed down with a few c.c. of absolute alcohol. The bottles were placed in the centrifuge and rotated until the solid matter formed a compact mass. The clear liquids were decanted into labeled flasks. The above treatment with absolute alcohol was repeated five times, using each time 200 c.c. of alcohol. The residues were washed twice, using each time 100 c.c. of absolute alcohol, by placing on the shaker for one hour, centrifuging and decanting the liquid. Each of the combined alcohol extracts was filtered by gravity through Whatman filters No.1, using four inch funnels. The flasks and filters were washed with absolute alcohol. The filtrates were made slightly acid with sulphuric acid and the alcohol recovered by distillation in Claissen flasks. Total nitrogen determin-

ations were made on each of the absolute alcohol extract residues.

The solid matter collected on the filters was added to the residues insoluble in absolute alcohol and the latter treated as described below in (C).

C. EXTRACTION WITH 1.0 PER CENT TRICHLORACETIC ACID

The residues insoluble in absolute alcohol were treated with 200 c.c. of 1.0 per cent trichloracetic acid. The bottles were placed on the ~~centrifuge~~ shaker as before, the time of extraction during the day being 7 to 8 hours and that during the night 14 to 15 hours. After each extraction the sides of the bottles were washed down with a few c.c. of water. The bottles were placed in the centrifuge and rotated until the solid matter formed a compact mass. The clear liquids were carefully decanted, avoiding as far as possible the decantation of any undissolved material. The residues were then treated six times with 1.0 per cent trichloracetic acid, using each time 200 c.c. The residues were then washed by treating each with 100 c.c. of ammonia free water, placing the bottles on the shaker for one hour, centrifuging, and decanting the liquid. This washing was continued until the residues were practically free from acid. Since reasonably clear decantates were obtained, they were not filtered as a small amount of undissolved material was found to interfere in no way with the future work.

The residues insoluble in 1.0 per cent trichloracetic acid were treated as described below in (D) and the four extracts as described below in (G).

D. EXTRACTION WITH DILUTE SODIUM HYDROXIDE SOLUTION

The residues insoluble in 1.0 per cent trichloracetic acid were each treated with 200 c.c. of alkali solution. The time of the extraction during the day was 7 to 8 hours, using 0.2 per cent sodium hydroxide solution, and the time of extraction over night was 14 to 15 hours using 0.1 per cent so-

dium hydroxide solution. After each extraction the sides of the centrifuge bottles were washed down with ammonia free water, the bottles placed in the centrifuge and rotated until the solid matter formed a compact mass. Decantation was carried out as before. Six extractions in all were made with dilute alkali. The residues were washed with 100 c.c. portions of ammonia free water, using the shaker and centrifuge as described above in (C) until practically free from alkali. The decantates were acidified as soon as they were poured off, using about 5 c.c. of concentrated hydrochloric acid for this purpose. When possible, concentration was begun immediately, otherwise the decantates were boiled every second day for a few minutes in order to prevent decomposition.

The residues insoluble in dilute sodium hydroxide solution were treated as described below in (E) and the alkali extracts as described below in (H).

E. EXTRACTION WITH TWENTY PER CENT HYDROCHLORIC ACID

The residues insoluble in dilute alkali were transferred to 500 c.c. round bottom digestion flasks, using 250 c.c. of twenty per cent hydrochloric acid to effect the transfer. The flasks were placed on wire gauze, the liquid heated to boiling and boiled for three minutes. The liquid was cooled to room temperature and filtered through hard paper on Buchner funnels by suction. The above treatment was repeated once. The residues were washed two or three times with 20 per cent hydrochloric acid, and then with ammonia free water until the residues were almost free from acid. The washings with water were collected separately, evaporated on the steam bath to a small volume and an equal volume of concentrated hydrochloric acid added to them before combining them with the 20 per cent hydrochloric acid extract.

The residues insoluble in 20 per cent hydrochloric acid were treated as described below in (F), and the acid extracts as described below in (I).

F. EXTRACTION WITH STRONG SODIUM HYDROXIDE SOLUTION

The residues insoluble in 20 per cent hydrochloric acid were transferred to the original 500 c.c. centrifuge bottles using ammonia free water to effect the transfer. The bottles were centrifuged and the liquids decanted. Fifty c.c. of 5 per cent sodium hydroxide solution were added to each centrifuge bottle. The bottles were placed on the shaker and allowed to remain for a period of 24 hours. The sides of the bottles were washed down with water, the bottles centrifuged and the extracts decanted as usual. This treatment was repeated twice, using each time 50 c.c. of 5 per cent sodium hydroxide solution. The residues were washed with ammonia free water using the shaker and centrifuge as described above, until they were nearly free from alkali.

The extracts combined with the washings were acidified with hydrochloric acid and concentrated in vacuo to a volume of about 150 c.c. The extracts were then transferred to round bottom digestion flasks, using an equal amount of concentrated hydrochloric acid to effect the transfer, and treated as described below in (J).

The residues insoluble in strong sodium hydroxide solution were transferred to Kjeldahl flasks and total nitrogen determined.

G. TREATMENT OF THE 1.0 PER CENT TRICHLORACETIC ACID EXTRACTS

The trichloracetic acid extracts, each of which measured about 2,000 c.c. were treated as follows; The solutions were made slightly alkaline with a dilute solution of sodium hydroxide solution, and then slightly but distinctly acid with dilute hydrochloric acid. The solutions were heated to boiling and 10 c.c. of Merck's colloidal ferric hydroxide (containing 5 per cent of Fe_2O_3) added drop by drop during vigorous boiling of the solution. The solution was boiled for one minute and then 3 c.c. of a solution

of crystallized magnesium sulphate (made by dissolving $MgSO_4 . 7H_2O$ in an equal volume of water) were added to coagulate the excess of colloidal ferric hydroxide. The solution was boiled again for one minute, and allowed to stand until the precipitate settled. Without filtering, precipitation was carried out twice more in the manner just described. The precipitate was allowed to settle and the solution filtered while still hot, using a four inch Buchner funnel. Usually it was not necessary to filter more than once in order to obtain a clear filtrate. The filtration was made with gentle suction at first, gradually increasing this when filtering the last one third of the solution. The precipitate was washed thoroughly with hot, ammonia free water, but the precipitate was not allowed to be sucked dry.

The clear filtrates were made up to a volume of 2,500 or 3,000 c.c., depending upon the amount of the filtrate, and total nitrogen determinations made in triplicate upon three portions of 250 c.c. each.

The colloidal iron precipitates were transferred to round bottom digestion flasks by means of twenty per cent hydrochloric acid, and treated as below in (K).

In the case of the samples C1, C2, C3, and C4, 1,500 c.c. of the clear filtrate, after having been made up to 3,000 c.c., were taken for reprecipitation by colloidal ferric hydroxide. This portion of the filtrate was precipitated twice, the solution filtered and the precipitate washed just as described above. The filtrate and washings were made up to definite volume and total nitrogen determined in three 250 c.c. portions. The precipitates were combined with the precipitates obtained in the first precipitation.

H. TREATMENT OF THE DILUTE SODIUM HYDROXIDE EXTRACTS OBTAINED ABOVE IN (D)

The dilute alkali extracts, after having been acidified with hydrochloric acid, were each concentrated in vacuo to about 150 c.c. The concentrated

liquids including the precipitated proteins were transferred to digestion flasks with an equal volume of concentrated hydrochloric acid, and completely hydrolyzed by boiling on a combined electric hot plate and sand bath for 15 hours, using reflux condensers. The completely hydrolyzed proteins were treated as described below in (L).

I. TREATMENT OF THE TWENTY PER CENT HYDROCHLORIC ACID EXTRACTS

The 20 per cent hydrochloric acid extracts were completely hydrolyzed by boiling for 15 hours on the hot plate, and were treated further as described below in (L).

J. TREATMENT OF THE EXTRACTS OBTAINED WITH 5 PER CENT SODIUM HYDROXIDE SOLUTION

The 5 per cent sodium hydroxide extracts were completely hydrolyzed by boiling for 15 hours on the hot plate as described above, and treated further as described below in (L).

K. TREATMENT OF PROTEINS PRECIPITATED BY COLLOIDAL IRON

The proteins precipitated by colloidal iron were completely hydrolyzed on the hot plate and treated further as described below in (L).

L. DETERMINATION OF HUMIN AND AMMONIA NITROGEN

DETERMINATION OF THE INSOLUBLE HUMIN NITROGEN. The completely hydrolyzed protein solutions from (H), (I), (J) and (K) above were filtered through the same filter. The residue was washed with hot ammonia free water, and trasnferred to round bottom digestion flasks, using about 250 c.c. of 0.1 per cent hydrochloric acid to effect the transfer. The residue was boiled over night on the hot plate, using reflux condensers. After filtering,

the residue was returned to the flask with 250 c.c. of 0.1 per cent hydrochloric acid and boiled for one hour. The residue was washed thoroly with hot, ammonia free water and transferred to a Kjeldahl flask for total nitrogen determination.

CONCENTRATION OF THE FILTRATES FROM THE INSOLUBLE HUMIN. The filtered solution and the washings from the insoluble humin were placed in a double necked distilling flask and concentrated under diminished pressure until all the hydrochloric acid possible had been driven off. The solution was concentrated almost to dryness, the sides of the flask washed down with ammonia free water and concentrated again almost to dryness. The solution was transferred to a 200 c.c. volumetric flask with ammonia free water and made up to the mark. This in turn was transferred to a clean double necked distilling flask by means of 100 c.c. of 95 per cent alcohol for determination of ammonia as indicated below.

DETERMINATION OF AMMONIA (AMID NITROGEN). For the determination of ammonia, a one liter double necked distilling flask and an ordinary one liter distilling flask were arranged as shown by Van Slyke (Fig. 1, Jour. Biol. Chem. Vol.10, p. 21, 1911). Ten per cent calcium hydrate suspension was added until the solution became alkaline and then 20 c.c. in excess were added. The apparatus was evacuated to a pressure of about 30 millimeters and the double necked distilling flask heated in a water bath at a temperature of 45 to 50 degrees C. The solution was distilled for one half hour or until it began to foam considerably. The amount of tenth normal acid added to the larger flask was 60 c.c. and to the trap 30 c.c.

When the distillation was finished, the flask was removed from the water bath, and the vacuum released by first allowing a small stream of air to enter through the side arm and then throughthe the capillary tube, and finally

opening the stopcock. The standard acid from the receiving flask and the smaller trap flask was washed into a half liter titrating flask and titrated back with standard sodium hydroxide solution, using alizarine sulphonate as indicator.

SOLUBLE HUMIN NITROGEN. During the distillation described above all of the black coloring matter present as soluble humin is adsorbed by the undissolved lime. The solution was filtered through hard paper on a 3 inch Buchner funnel using suction. The insoluble residue was washed thoroughly with ammonia free water and transferred to a round bottom digestion flask with about 250 c.c. ammonia free water. The flask was placed on the sand bath and the residue boiled for 15 minutes. After filtering and washing thoroughly as before, the residue was transferred to a Kjeldahl flask for total nitrogen determination, using a portion of sulphuric acid to wash off the Buchner funnel. The filtrate and washings from the soluble humin were then treated further as described below.

M. CONTINUATION OF THE VAN SLYKE METHOD. ANALYSIS OF THE BASES, DETERMINATION OF AMINO NITROGEN IN FILTRATE FROM THE BASES; ETC.

The filtrate and washings from the soluble humin were neutralized with hydrochloric acid, returned to vacuum distilling flasks and concentrated to about 65 c.c. The solution was washed into a 250 c.c. volumetric flask and made up to the mark. Two portions of 100 c.c. each were then transferred to 300 c.c. Erlenmeyer flasks which had been marked to indicate a volume of 200 c.c., the balance of the solution being discarded. In the case of samples C5, C6, C7 and C8, a different procedure was utilized. With these samples, the solutions were made up to 200 c.c., and the solutions were then divided into equal portions of 100 c.c. each by first removing 100 c.c. by means of a pipette and then washing the remainder of the solution, including that adhering to the inside of the pipette, into another Erlenmeyer flask.

Eighteen cubic centimeters of concentrated hydrochloric acid were then added to each portion of the solution. Up to this point, the analysis of the four samples had proceeded simultaneously, but after dividing each of the samples into two portions and adding the concentrated hydrochloric acid, as indicated just above, one half of the samples were allowed to stand until the analysis of the other four portions had been nearly completed.

PRECIPITATION AND WASHING OF THE BASES (CYSTINE, LYSINE, ARGININE AND HISTIDINE). A concentrated solution of 15 grams of phosphotungstic acid in water was added drop by drop from a pipette to each solution with constant shaking. In the case of samples C7 and C8, an improved procedure developed in this laboratory was utilized, namely, the addition of the acidified sample to the phosphotungstic acid solution. The phosphotungstic acid used in the precipitation and in the washing solution was purified according to the modified Winterstein method as described below. Each of the entire solutions was then diluted with water up to 200 c.c. and heated on the steam bath until the precipitate of the bases was nearly dissolved. The solutions were heated very slowly, first by placing the flasks upon the top of the covered steam bath for a half hour or more, then by placing in the boiling water for one half minute, removing and shaking and repeating this procedure until the solutions had gradually come up to the temperature of the bath. The flasks were then allowed to remain in the water as long as any of the precipitate continued to disselve. The flasks were removed from the steam bath and the solutions allowed to stand 48 hours to permit complete precipition of the bases. On cooling, the bases reprecipitated as crystalline or granular phosphotungstates which could be readily washed and filtered.

In order to completely separate the precipitate of the bases from the unprecipitated amino acids, the solutions and precipitates were poured into

a two inch Buchner funnel which had been fitted with an accurately cut, hardened filter paper. The mother liquors were drawn off as completely as possible by a steady and moderately strong suction, the precipitates being pressed down by a flattened rod. The precipitates were not allowed to be sucked dry during filtration and washing, but suction was maintained throughout the procedure. Eight portions of 10 c.c. each of a washing solution containing 3.5 per cent hydrochloric acid and 2.5 per cent phosphotungstic acid were used in washing each precipitate, the solution being delivered onto the precipitate in a fine stream from a pipette, while the precipitate was stirred and the lumps broken by means of a flattened stirring rod. The first two to three portions of the wash solution were used to rinse the flask in which the precipitation was carried out, without, however, removing all of the particles of the precipitate. The wash solution was cooled to 0 degrees C before being used.

DECOMPOSITION OF THE PHOSPHOTUNGSTIC ACID PRECIPITATE BY A MIXTURE OF ETHER AND AMYL ALCOHOL. The precipitate of the bases, after having been washed as described above, was transferred to a 500 c.c. separatory funnel by means of a spatula and washing, from 200 to 300 c.c. of ammonia free water being used to effect the transfer. After removing the precipitate as completely as possible by mechanical means, the filter paper was spread out on the bottom of a dish and washed with water made alkaline with a few drops of sodium hydroxide solution in order to dissolve any particles of the precipitate imbedded in the fibers of the filter paper. Since the Erlenmeyer flask in which the precipitation was carried out contained some particles of precipitate, these were washed out as completely as possible and the flask rinsed with the alkaline wash water. The Buchner funnel used in filtration was also rinsed with the same wash water. These washings were neutralized with dilute hydrochloric acid before they were added to the main portion.

Seven and one half c.c. of concentrated hydrochloric acid and 100 c.c. of a solution of equal volumes of amyl alcohol and ether were added to the mixture, which was then shaken for about one minute longer than was necessary to effect complete solution of the precipitate. Since the aqueous and alcohol-ether layers did not separate readily on account of humin unadsorbed by the calcium hydrate, the entire mixture was filtered by the use of a 2 inch Buchner funnel and suction. The residue was washed several times with alternate portions of ammonia free water and alcohol-ether. The residue together with the filter paper was transferred to a Kjeldahl flask and total nitrogen determined. The filtrate was returned to the separatory funnel, and after the two layers had separated, the lower aqueous layer was drawn off into another 500 c.c. separatory funnel. The aqueous layer was then extracted with three more portions of alcohol-ether,, using about 50 c.c. each time. Finally the combined alcohol-ether extracts were shaken out once or twice with water to remove traces of bases that might have been carried into the extract. This water extract, after separation from the alcohol-ether, was shaken out with about 50 c.c. of fresh amyl alcohol-ether, and added to the main portion of the solution of the bases.

The amyl alcohol-ether extract was transferred to a Kjeldahl flask, a few pieces of broken clay plate and a small amount of concentrated sulphuric acid added, and the ether driven off by allowing the flask to stand on the steam bath. The amyl alcohol was recovered by distillation, and total nitrogen determined in the residue.

The solution of the bases was concentrated to dryness under diminished pressure in order to drive off the free hydrochloric acid. About 25 c.c. of ammonia free water was added to the residue and all particles adhering to the inside of the flask completely loosened. The solution was then con-

centrated to a volume of about 10 c.c. The contents of the flask were filtered through a small quantitative filter, the filtrate being collected in a 50 c.c. volumetric flask. The distilling flask was washed repeatedly with portions of 5 to 8 c.c. of ammonia free water, and a bent glass rod used to remove all particles adhering to the sides of the flask. The filter was washed thoroughly and the filtrate and washings made up to a volume of 50 c.c. The residue, together with the filter paper, was transferred to a Kjeldahl flask and total nitrogen determined. Four of these solutions of the bases were thus available for analysis at approximately the same time.

PURIFICATION OF THE PHOSPHOTUNGSTIC ACID BY THE METHOD OF WINTERSTEIN (32). This method, with its modifications as employed in this laboratory, is as follows;- 15 grams of phosphotungstic acid, 50 c.c. of 0.2 per cent hydrochloric acid and 50 c.c. of ether are placed in a separatory funnel and shaken until the solid phosphotungstic acid has completely dissolved. On standing for about five minutes three layers separate. The clear lower layer, consisting of phosphotungstic acid in ether, is carefully drawn off into a casserole or evaporating dish. One or two drops of concentrated hydrochloric acid are added to the contents of the funnel in order to break up any emulsion that had not been completely broken up by previous treatment, and the mixture shaken. After standing, the ether-phosphotungstic acid layer, if one forms, is again drawn off into the casserole or evaporating dish. This procedure is repeated until no more of this layer is obtained. The remaining two layers in the separatory funnel are employed for the purification of a second 15 gram portion of phosphotungstic acid.

The ethereal solution is allowed to evaporate on the steam bath at a low temperature until a thick syrup remains. The dish is removed from the heat, sunlight being avoided, and the remaining ether allowed to evaporate spon-

taneously with the production of crystals of purified phosphotungstic acid. The latter process is hastened by blowing a current of air from an electric fan over the dish. The liquors from the purification are saved so that the small amount of phosphotungstic acid remaining in them may be later recovered.

DETERMINATION OF ARGININE. The determination of arginine is based upon the principle that arginine, when boiled with alkali, evolves half of its nitrogen in the form of ammonia.

Twenty five c.c. of the 50 c.c. of the solution of the bases were placed in a 500 c.c. Kjeldahl flask of the apparatus as shown in Fig. 2, Jour. Biol. Chem. 10, p. 26. The Folin bulb at the top of the condenser was connected by means of a tightly fitting rubber stopper. For the first determinations 15 c.c. of standard acid were placed in the Folin bulb, and for the later determinations, on account of the high arginine content of cottonseed meal, 20 c.c.. A small current of air, purified by passing through strong alkali and sulphuric acid solutions, was forced through the apparatus during the entire distillation. The air was admitted to the apparatus by means of small capillary tubes placed in the rubber stoppers of the Kjeldahl flasks and extending well below the surface of the solution. After gentle boiling for 5 1-2 hours, the water supply was shut off and five minutes later the water removed from the condensers. At the end of six hours the solution in the Folin bulb was washed into a titrating flask and the acid titrated back with the use of alizarin sulphonate as indicator. Two determinations were always made simultaneously.

DETERMINATION OF AMINO NITROGEN OF THE BASES (CYSTINE, LYSINE, ARGININE AND HISTIDINE.) This determination was carried out as directed by Van Slyke (8), using one portion of 5 c.c. of the solution originally amounting to 50 c.c. The determinations were carried out over a period of 30 minutes,

and blank determinations were made with the reagents, using 5 c.c. of ammonia free water instead of the solution of the bases, for the same length of time. In most cases but a single determination was made with each sample, but when the results of the different samples did not agree well with each other, duplicate determinations were made.

DETERMINATION OF THE TOTAL NITROGEN OF THE BASES: One portion of 5 c.c. of each of the solutions of the bases was used for this determination. The determination was carried out in the usual manner with the exception of the preliminary digestion with sulphuric acid, which was continued for six hours after the solutions became clear. Usually four of the solutions of the bases were available for this determination at approximately the same time.

DETERMINATION OF CYSTINE. The method employed for this determination was that based on Benedict's principle of oxidation of the organic sulphur by ignition with copper nitrate. For this determination 10 c.c. of the solution of each of the bases were usually employed, but in those cases in which it was necessary to repeat either the amino nitrogen or total nitrogen determinations, the portions of the solutions of the two samples which were obtained originally from the same sample of cottonseed meal, which remained after having made the above determinations, were combined to form a composite sample for the cystine determination.

A measured portion of the solution containing the bases was transferred to fused silica dishes of 7 to 10 cm. diameter and 10 c.c. of Denis' modification of Benedict's solution added. About 0.1 gram pure sucrose was added and the mixture concentrated to dryness on the steam bath. It was then heated gently in an air bath and finally over a free flame, gradually increasing the temperature until the mixture was thoroughly charred. The dish was placed in a muffle furnace heated to a dull red heat and allowed to remain for 15 minutes. After cooling, 10 c.c. of 10 per cent hydrochloric acid were added

and the charred mass dissolved by warming and stirring. The solution was filtered through a good grade of filter paper and the filter washed thoroughly with hot water. The solution was diluted to about 150 c.c. and heated to boiling. Ten c.c. of a 5 per cent solution of barium chloride were added drop by drop while the solution was boiled vigorously. After standing for two days, the precipitate which formed was filtered off on a weighed Gooch crucible, washed until free from chlorides and dried over night in an oven at 100 degrees C. The crucible was brought to constant weight by repeated heating in a muffle furnace, and the weight of the barium sulfate determined.

Blank determinations with the reagents were made simultaneously with those of the bases, using 10 c.c. of ammonia free water in place of the solution of the bases. By the use of carefully purified sodium chloride used in making up Denis' reagent and the employment of fused silica dishes, much lower blanks were obtained than when procelain dishes were used. The blanks were also much more constant in amount.

DETERMINATION OF THE TOTAL NITROGEN IN THE FILTRATE FROM THE BASES. Sodium hydroxide solution was added to the combined filtrate and washings from the phosphotungstic acid precipitate until the solution became slightly turbid by the formation of a precipitate and was almost neutral to litmus paper. On adding a little more alkali solution, the solution became clear. It was then slightly acidified with acetic acid. The solution was placed in a Claissen distilling flask and concentrated under reduced pressure until the salts began to crystallize out. The contents of the flask were then washed into a 150 c.c. measuring flask and diluted up to the mark. The solution was allowed to stand over night and then filtered through a dry filter and a dry funnel into a dry flask. The residue was thoroughly washed with ammonia free water, the washings being discarded, and total nitrogen determined in the residue together with the filter paper. Duplicate portions of 50 c.c.

each of the solution were taken for total nitrogen determination. For the digestion of each portion, 15 grams of sodium sulfate, 35 c.c. of concentrated sulphuric acid and a small amount of mercury were used. The sulphuric acid was added slowly while cooling the flask in running water on account of the rapid evolution of hydrochloric acid gas. The digestion was continued for six hours after the solutions had become clear, in order to prevent interference of the phosphotungstic acid in the determination.

DETERMINATION OF AMINO NITROGEN IN THE FILTRATE FROM THE BASES. This procedure was carried out essentially as directed by Van Slyke (8). The determinations were made in triplicate, using 10 c.c. portions of the filtrate from the bases, and employing the large sized gas burette. Blank determinations were also made with the reagents, using 10 c.c. of ammonia free water in place of the sample. In all cases the determinations were continued for six minutes with continuous shaking.

III. DISCUSSION OF THE RESULTS OF THE CHEMICAL ANALYSIS OF THE PROTEINS OF COTTONSEED MEAL

GENERAL DISCUSSION. The results obtained by application of the method of chemical analysis outlined in the preceding section to eight portions of the same original sample of cottonseed meal are presented in detail in Tables 1 to 21, inclusive, of the Appendix. Summaries of these results are shown in the accompanying Tables 1 and 2. Table 1 shows the values expressed in per centage of the total nitrogen present in the sample of feedingstuff when it was taken for analysis, while Table 2 shows the same values expressed in percentage of the feedingstuff itself.

Two averages are included in the tables. The first is compiled by taking the average of all values obtained by analysis of the entire eight samples. The second average is obtained by averaging the results secured in the analysis of the complete samples C2, C3, C6 and C7. It is believed that the latter average more nearly expresses the actual composition of the commercial cottonseed meal used, for the following reasons; (a) These samples, i.e., C2, C3, C6 and C7, show the best agreeing results throughout. The two parts of sample C1 agree well in the amount of arginine nitrogen, but show a considerable difference in the amounts of amino nitrogen, non-amino nitrogen and histidine nitrogen. The totals of the nonprotein nitrogen plus the protein nitrogen are considerably below the average of all the samples. In the sample C4 the non-amino nitrogen is particularly low, this value being one of the principal factors contributing to the noticeably low nonprotein plus protein of this sample. Sample C5 is omitted from the average partly on account of the non-agreement of its arginine nitrogen and histidine nitrogen values. Of the latter values, one is 3 per cent above the average of all samples. (b) The second average, i.e., of samples C2, C3, C6 and C7, includes values which are

TABLE 1.- SUMMARY OF THE RESULTS OF ANALYSIS OF THE PROTEINS OF COTTONSEED MEAL
(Results expressed in percentage of the total nitrogen in sample)

	Nonprotein Nitrogen				Results of the Van Slyke Analysis										Uncharacterized Nitrogen Lost in Method of Analysis				Total		
Sample No.	Soluble in absolute ether	Soluble in absolute alcohol	In filtrate from colloidal iron	Total nonprotein nitrogen	Insoluble humin nitrogen	Soluble humin nitrogen	Ammonia nitrogen	Arginine nitrogen	Cystine nitrogen	Histidine nitrogen	Lysine nitrogen	Amino nitrogen in filtrate from the bases	Non-amino N in filtrate from the bases	Nonprotein N + results of Van Slyke analysis	Insoluble in strong alkali	Unadsorbed humin (filtered from sol. during decomposition of phosphotungstate ppt.)	Soluble in amyl alcohol ether mixture	In residue from solution of the bases	In residue from solution of the filtrate from the bases	Total nitrogen lost	Total nitrogen accounted for
C1	0.021	0.570	4.943[2]	5.534	2.609	3.462	9.455	18.569 18.775	0.961 0.961	6.095 4,876	3.871 4.609	38.495 41.466	4.079 2.463	93.130 94.210	0.220	2.403 2.145	0.906 1.043	0.206 0.206	0.017 0.055	3.752 3.669	96.992 97.879
C2	0.089	0.618	4.870[2]	5.577	2.609	5.117	9.689	18.981 19.119	0.821 0.983	5.622 7.038	3.625 2.494	39.646 41.432	3.908 2.955	95.595 97.013	0.260	2.042 1.081	0.734 1.078	0.103 0.326	0.027 0.051	3.166 2.796	98.761 99.809
C3	0.202	0.652	5.053[2]	5.907	2.492	5.459	9.929	18.295 18.638	1.035 1.101	7.966 7.120	3.471 3.668	39.157 38.546	2.348 1.454	96.062 94.314	0.302	1.867 1.448	0.614 0.961	0.240 0.200	0.079 0.133	3.102 3.044	99.164 97.358
C4	0.109	0.614	5.531[1]	6.254	2.628	4.477	8.892	19.160 17.635	1.285 0.961	6.441 8.038	4.430 4.510	39.828[a] 39.828	0.161 0.161	93.551 93.379	0.233	3.011 3.076	0.841 1.428	0.243 0.247	0.130[a] 0.130	4.458 5.144	98.009 98.493
C5	0.081	0.506	5.245[1]	5.832	2.981	2.415	9.249	18.701 16.339	1.051 1.051	6.987 10.181	5.042 3.881	41.659 42.257	2.784 2.577	96.701 96.763	-----[b]	1.230 1.153	0.645 0.736	0.255 0.601	0.217 0.082	2.777 3.002	99.478 99.765
C6	0.129	0.489	5.722[1]	6.340	2.930	2.650	9.318	19.580 19.305	0.963 0.933	6.568 6.163	4.780 5.216	41.659 42.141	2.504 2.076	97.292 97.072	0.685	1.021 1.016	0.642 0.917	0.445 0.315	0.096 0.096	2.889 3.029	100.818 100.101
C7	0.081	0.420	6.097[1]	6.598	2.763	2.334	9.002	17.932 18.041	0.692 0.722	9.627 9.074	3.503 3.697	39.131 39,276	2.921 3.253	94.503 94.760	0.719	0.818 0.725	0.994 1.057	0.068 0.124	0.054 0.076	2.653 2.701	97.156 97.461
C8	0.047	0.506	7.012[1]	7.564	2.772	2.746	9.764	19.799 20.404	0.810 0.751	5.657 7.271	5.845 4.702	43.023 43.939	3.095 3.812	101.076 103.726	0.589	1.384 1.343	1.049 1.087	0.151 0.115	0.054 0.082	3.227 3.216	104.303 106.942
Aver.[3]	0.095	0.547	5.559[1]	6.201	2.772	3.582	9.412	18.705	0.943	7.170	4.209	40.718	2.535	96.197	0.430	1.610	0.921	0.240	0.086	3.287	99.484
Aver.[4]	0.125	0.545	5.436[1]	6.106	2.699	3.890	9.485	18.736	0.906	7.397	3.807	40.124	2.677	95.827	0.492	1.252	0.875	0.228	0.076	2.923	98.750

a Determination lost. Value from other half of sample substituted
b Determination lost. Average of 7 determinations used.
1 After first precipitation
2 After second precipitation
3 Average of all determinations
4 Average of complete samples, C2, C3, C6, C7

TABLE 2.- SUMMARY OF THE RESULTS OF ANALYSIS OF THE PROTEINS OF COTTONSEED MEAL
(Results expressed in percentage of the feedingstuff)

	Nonprotein Nitrogen								Results of the Van Slyke Analysis						Uncharacterized Nitrogen Lost						Total
Sample No.	Soluble in ether	Soluble in absolute alcohol	In filtrate from colloidal iron	Total nonprotein nitrogen	Insoluble humin nitrogen	Soluble humin nitrogen	Ammonia nitrogen	Arginine nitrogen	Cystine nitrogen	Histidine nitrogen	Lysine nitrogen	Amino nitrogen in filtrate from bases	Non-amino nitrogen in filtrate from bases	Nonprotein N + results of Van Slyke analysis	Insoluble in strong alkali	Unadsorbed humin (filtered from sol. during decomp. of phosphotungstate ppt.)	Soluble amyl-alcohol ether	Residue from solution of bases	Residue from solution of filtrate of bases	Total nitrogen lost	Total nitrogen accounted for
C1	0.0041	0.0387	0.3660[2]	0.3761	0.1769	0.2352	0.6426	1.2620 1.2760	0.0653 0.0653	0.4142 0.3814	0.2631 0.3133	2.6157 2.8180	0.2773 0.1674	6.3284 6.4022	0.0150	0.1633 0.1458	0.0616 0.0709	0.0140 0.0140	0.0012 0.0037	0.2551 0.2494	6.5835 6.6516
C2	0.0061	0.0420	0.3810[2]	0.3790	0.1773	0.3478	0.6585	1.2900 1.2993	0.0558 0.0668	0.3821 0.4783	0.2464 0.1695	2.6940 2.8153	0.2656 0.2009	6.4966 6.5928	0.0177	0.1388 0.0735	0.0499 0.0783	0.0070 0.0222	0.0019 0.0035	0.2153 0.1902	6.7119 6.7830
C3	0.0137	0.0443	0.3435[2]	0.4015	0.1694	0.3710	0.6748	1.2433 1.2666	0.0703 0.0748	0.5414 0.4839	0.2360 0.2493	2.6610 2.6193	0.1596 0.0988	6.5283 6.4094	0.0205	0.1269 0.0985	0.0418 0.0653	0.0163 0.0136	0.0054 0.0091	0.2109 0.2070	6.7392 6.6164
C4	0.0075	0.0417	0.3759[1]	0.4250	0.1783	0.3043	0.6043	1.3021 1.1985	0.0873 0.0653	0.4377 0.5462	0.3011 0.3065	2.7067[a] 2.7067	0.0110 0.0110	6.3579 6.3462	0.0158	0.2046 0.2091	0.0572 0.0971	0.0165 0.0168	0.0089[a] 0.0089	0.3030 0.3437	6.6609 6.6939
C5	0.0055	0.0345	0.3563[1]	0.3963	0.2026	0.1641	0.6286	1.2709 1.1104	0.0715 0.0715	0.4749 0.6919	0.3427 0.2638	2.8310 2.8715	0.1892 0.1751	6.5718 6.5758	------[b]	0.0836 0.0784	0.0439 0.0500	0.0178 0.0409	0.0147 0.0056	0.1887 0.2041	6.7605 6.7799
C6	0.0088	0.0332	0.3889[1]	0.4309	0.1991	0.1801	0.6333	1.3300 1.3120	0.0655 0.0635	0.4464 0.4188	0.3249 0.3545	2.8310 2.8630	0.1702 0.1411	6.6120 6.5963	0.0465	0.0694 0.0691	0.0437 0.0623	0.0302 0.0215	0.0065 0.0065	0.2163 0.2059	6.8083 6.8022
C7	0.0055	0.0285	0.4144[1]	0.4484	0.1878	0.1586	0.6118	1.2186 1.2260	0.0471 0.0491	0.6543 0.6167	0.2381 0.2513	2.6590 2.6690	0.1986 0.2211	6.4223 6.4398	0.0489	0.0556 0.0493	0.0675 0.0719	0.0047 0.0084	0.0037 0.0052	0.1804 0.1837	6.6028 6.6235
C8	0.0032	0.0344	0.4765[1]	0.5140	0.1884	0.1866	0.6635	1.3456 1.3866	0.0551 0.0511	0.3845 0.4942	0.3972 0.3196	2.9235 2.9860	0.2104 0.2591	6.8689 7.0492	0.0400	0.0941 0.0913	0.0713 0.0739	0.0103 0.0078	0.0037 0.0056	0.2194 0.2186	7.0883 7.2678
Aver.[3]	0.0065	0.0372	0.3778[1]	0.4214	0.1849	0.2434	0.6396	1.2712	0.0641	0.4873	0.2861	2.7669	0.1723	6.5374	0.0292	0.1095	0.0626	0.0163	0.059	0.2235	6.7609
Aver.[4]	0.0085	0.0370	0.3695[1]	0.4150	0.1834	0.2644	0.6446	1.2733	0.0616	0.5027	0.2588	2.7265	0.1820	6.5123	0.0334	0.0851	0.0595	0.0155	0.052	0.1987	6.7110

1 After first precipitation
2 After second precipitation
3 Average of all determinations
4 Average of complete samples C2, C3, C6, C7
a Determination lost. Value for other half of sample substituted
b Determination lost. Average of seven determinations used.

most free from obvious errors. In making the determinations in the case of sample C4, two determinations were lost, and in the case of sample C5, one determination was lost. While the results obtained for sample C8 agree fairly well throughout with themselves and with the average, the results are consistently high, and it is excluded from this average on the grounds of the totals obtained, which are obviously too high.

NONPROTEIN NITROGEN. The first section of Tables 1 and 2 shows the amount of nitrogen removed in the preliminary extractions with absolute ether, absolute alcohol and trichloracetic.acid. While the absolute ether in the cold is used primarily to remove the lipins, such as the oils, waxes, etc., it also dissolves various amounts of other substances, such as coloring matters, and at the same time a small amount of nitrogen. The thorough extraction with absolute alcohol following the treatment with absolute ether presumably completes the extraction initiated by ether. The alcohol removes somewhat more nitrogen than the extraction with ether. The bulk of the nonprotein nitrogen accounted for, about 89 per cent of the total, remains, however, in the trichloracetic acid extracts after precipitation of the proteins by colloidal ferric hydrate and the removal of the precipitate by filtration. That the nitrogen determined in these latter extracts is not protein nitrogen is apparent from the work of Van Slyke, Vinograd, Vilchur and Losee (35), Hill (36), Wolff (37), and others.

It seems from the study of the character of the nonprotein nitrogenous constituents of feedingstuffs by Grindley and Eckstein (38) that the forms of nitrogen represented in this classification consist principally of those forms naturally resulting from the cleavage of the proteins upon hydrolysis and therefore could not interfere in the determination of the characteristic chemical groups of the proteins were they not removed in the preliminary extractions. Neidig and Snyder (39), who recently determined the proportion of

nitrogen in the form of ammonia in the ether extracts and alcohol extracts of different kinds of silage, found that from 28.1 per cent to 100 per cent of the ether extract nitrogen consists of ammonia nitrogen, while from 14.2 per cent to 23.4 per cent of the alcohol extract nitrogen is yielded as ammonia nitrogen. This indicates that only a part of the nitrogen soluble in ether and alcohol would appear in the ammonia fraction were it not removed previous to hydrolysis. Therefore, the removal of the nonprotein nitrogen at this point avoids possible complications in the further prosecution of the analytical procedure, and it is believed that the accuracy of the further determinations has been increased over that of previous methods by the removal of the nonprotein nitrogen before hydrolysis of the proteins. The total amount of the nonprotein nitrogen present in cottonseed meal found by the method used, amounted to 6.106 per cent of the total nitrogen contained in the feedingstuff.

RESULTS OF THE VAN SLYKE ANALYSIS. It is a matter of common knowledge that one of the important sources of loss in the analysis of proteins by methods involving the employment of acid hydrolysis is the formation of an insoluble black substance called humin. The term melanin is also applied to this substance, on account of its supposed relationship or similarity to the naturally occuring body pigments. The amount of humin formed in acid hydrolysis of the proteins is greatly increased by the presence of carbohydrates, as shown by Gortner and associates (40,41), Hart and Sure (42), and Osborne, Van Slyke, Leavenworth and Vinograd (43). A part of the humin formed, however, remains in solution in the hydrochloric acid, and is termed soluble humin.

In these experiments the soluble humin which is adsorbed by the lime used in neutralizing the hydrolysate when determining ammonia nitrogen, carried with it a larger amount of nitrogen than the insoluble humin. The

TABLE 3.- AMOUNTS OF HUMIN NITROGEN IN PURE PROTEINS EXPRESSED IN PERCENTAGE OF THE TOTAL NITROGEN OF THE PROTEIN

Protein and description	Humin nitrogen per cent
Gliadin from wheat	0.86
Edestin	1.83
Fibrin (Merck's)	3.43
Oxyhemoglobin ("pure, crystallized")	3.60
Dog's hair	7.35

The amount of nitrogen recovered as ammonia was quite constant in all the samples. Little can be said in regard to the significance of this fraction, aside from the fact that the proportion of the total nitrogen of cottonseed meal which appears as ammonia is quite in harmony with that of other feedingstuffs.

A particularly characteristic feature of the amino acid content of cottonseed meal is the remarkably high content of arginine. This is much higher than that found in any other feedingstuff so far examined, with the exception of peanuts, altho it is not so high as that found in some other vegetable proteins. Van Slyke (8) found 27.05 per cent arginine nitrogen in edestin while Nollau reported that hemp seed, peanuts, black walnuts, and hickory nuts have an arginine nitrogen content of more than 20 per cent.

In the Van Slyke procedure the only amino acids determined by direct analysis are arginine and cystine. Just how much importance may be attached to the results obtained for the latter is questionable, even tho the values found in the different samples do not vary widely. These values, however, probably fall short of the true value, due to losses in the determination of cystine. Van Slyke (8) has shown that boiling cystine for 16 hours with hy-

drochloric acid resulted in the conversion of one half of its nitrogen into forms not precipitable by phosphotungstic acid. Since in the analytical procedure described above, hydrolysis of the proteins was carried out by boiling them with 20 per cent hydrochloric acid for 15 hours, it is probable that much of the cystine was destroyed during that reaction. If it is assumed that the correct value for cystine should be double that actually obtained, then the total nitrogen of the bases would amount to 31,75 per cent of the total nitrogen of the feedingstuff.

The values given for histidine and lysine are somewhat variable among the different samples. These variations are likely due in large measure to the indirect method used in their determination, since slight errors in any or all of the three direct determinations of arginine nitrogen, cystine nitrogen and the total nitrogen of the bases are doubtless allreflected at these points.

The content of mono-amino acid nitrogen of cottonseed meal is considerably less than that found in other feedingstuffs, possibly due to the greater proportion of the total nitrogen which is formed by the basic amino acids. One of the interesting features of the results of the Van Slyke analysis of the proteins of cottonseed meal is brought out in the summation of the ammonia nitrogen, the nitrogen of the bases, mono-amino acid nitrogen, and non-amino acid nitrogen, the four groups which represent the total content of strictly amino acid nitrogen as determined by this method. The sum of these is 83.132 per cent. While this sum is not so great as that in the case of some other feedingstuffs or of animal proteins, as determined by previous investigators employing the Van Slyke method of analysis, it is a much larger amount that it was possible to secure in most cases from comparable sources by the methods of isolation and purification employed by the earlier investigators. Thus the tabulations of Lusk (44), combining the results of Osborne

and associates in this field, show the maximum amino acid content of zein of maize, to be 88.87 per cent and that of gliadin of wheat as 85.68 per cent, but in the majority of cases the sum of the nitrogen content of the amino acids actually isolated from vegetable proteins ranges from 50 to 65 per cent of the total nitrogen of the protein.

UNCHARACTERIZED NITROGEN LOST IN ANALYSIS. In the various steps of the analytical procedure small amounts of nitrogen of unknown character are included in residues and solutions which are discarded. In general, these have been disregarded by workers in other laboratories, especially those losses occurring at points indicated in Table 1, by the last four of the subheadings included under the heading "Uncharacterized nitrogen lost in analysis", but in this laboratory the nitrogen discarded at each of these steps has been determined. Under the above mentioned headings, it is shown that, on the average, only 0.492 per cent of the total nitrogen remains in the residues insoluble in strong alkali, or in other words 99.508 per cent of the total nitrogen of the feedingstuff is extracted as a result of the method employed, and that in individual cases as much as 99.78 per cent of the total nitrogen present was removed. As previous workers failed to isolate the proteins from the feedingstuff before hydrolysis, it is not established that part of the insoluble residue discarded in their methods did not include some nitrogen in the form of non-hydrolyzed protein altho this does not seem highly probable. It is believed, however, that the nearly complete extraction of the protein before hydrolysis leads to the accuracy of the method by facilitating hydrolysis and in reducing the amount of humin.

The largest item of loss occurs in the residue which remains after dissolving the precipitate of the bases in the amyl alcohol-ether mixture. This, presumably, is soluble humin which has not been adsorbed by the lime in the determination of ammonia, and fouls the solution at this point. This difficulty

was also enccuntered by Menaul (45), who employed a preliminary precipitation with phosphotungstic acid in boiling solution for the separation of the humin and ammonia before the precipitation of the bases. In the present investigation, very little of the soluble humin appeared when the bases were precipitated, in most cases the precipitates being free from black particles. Washing with alternate portions of amyl alcohol-ether and water and then taking up the residue and washing thoroughly with water seemed to have little effect in reducing this source of loss. Acconsiderable portion of the nitrogen lost is soluble in the amyl alcohol-ether mixture, while smaller losses occur in the residues resulting from concentration of the solutions of the bases and filtrates from the bases. Presumably, the second, third and fourth items of loss include some nitrogen which should be credited to the bases, but the character of this nitrogen was not determined. If these losses can be reduced, the total nitrogen of the bases of cottonseed meal may be found to be somewhat greater than the amount here reported.

TOTAL NITROGEN ACCOUNTED FOR. Summation of the nitrogen found in the various fractions of the prctein molecule together with that in the unavoidable losses in the procedure gives totals which average 98.75 per cent. While the use of the Van Slyke method of analysis has enabled others to account for as great a proportion of the nitrogen of feedingstuffs, it is doubtful, for reasons pointed out below, if their results give as accurate a picture of the distribution of nitrogen in feedingstuffs as is obtained by the procedure employed in the present investigation.

PHYSIOLOGICAL SIGNIFICANCE OF THE BASIC AMINO ACIDS. Our knowledge of the physiological role of arginine and histidine has been enhanced by the studies of Ackroyd and Hopkins (46). Employing rations in which the nitrogen was provided in the form of hydrolyzed casein from which these two amino acids had been removed by precipitation according to the method of Kossel and

Kutcher, it was found that rats receiving these rations declined rapidly in weight, but that when either amino acid was returned to the ration, loss in weight was prevented and some growth ensued. The investigators suggest that possibly either of these amino acids may be converted into the other by the animal body. It was further observed that when arginine and histidine were removed from the ration, the excretion of allantoin, which is the main end product of purine metabolism in the rat, was lowered. Subsequent experiments proved that the falling off the allantoin excretion was not due to lowered metabolism. From these observations and from the fact that the arginine, histidine and guanine molecules have similar structural relationships, it was concluded that possibly one of the functions of arginine and histidine is to furnish the raw material for the purine metabolism of the animal organism.

The above conclusion regarding the importance of arginine in purine metabolism is given added weight by the findings of Myer and Fine (47) regarding the creatine content of muscle. Differences of as much as 2.5 per cent in the creatine content of muscle were noted as a result of feedings rations high and low in arginine.

That cystine plays an important part in nutrition has been brought out by several investigators, among them Osborne and Mendel (48). The latter obtained adequate growth by the addition of cystine to rations containing 9 per cent of casein, on which growth had been limited. Geiling (40), working in this laboratory, concluded that cystine seems to be necessary for the maintenance of adult mice. The importance of cystine to the animal organism is admirably set forth by Mathews (50).

"In the intermediary metabolism of the body, that is the metabolism of the tissue, sulphur probably plays a very important role. This is shown not only by the fact that it is absolutely necessary for the continued existence of the body, as necessary as nitrogen or any of the other elements, but also by the fact that it is one of the most labile elements of the protein mole-

cule. No other element is split off from the proteins with greater ease than this. It is, indeed, the labile element par excellence. Moreover, cystine, which is one of the amino acids, readily oxidizes itself. It is a reducing body. It oxidizes spontaneously and there are many points in its oxidation which strongly resemble the process of respiration. Thus the most favorable concentration of hydrogen ions for the oxidation of cysteine is the same as that in protoplasm; both cysteine and protoplasm are poisoned by many of the same substances, such as the nitriles, the cyanides, acids, and the heavy metals; their oxidation are catalyzed or hastened in the same manner by iron, arsenic and some other agents. For these reasons it has been suggested by Hefter and the author that there is more than a superficial connection between the oxidation of cysteine and the respiration of the cell."

The necessity of lysine for growth has been conclusively demonstrated by Osborne and Mendel (51). When gliadin of wheat, which contains only a minute amount of lysine, formed the sole source of protein in the rations of rats, the live weight of the animals was maintained over long periods, but normal growth could not be secured. When lysine was added to the rations, normal growth occurred. In other investigations (52), in which zein of maize was used as the source of the protein, it was found that a rat could be maintained at an almost constant weight of 50 grams for a period of 182 days when tryptophane was added to the extent of 3 per cent of the zein. The further addition of lysine induced normal growth. Further study (53) of the necessity of lysine in the ration convinced these investigators that about 2 per cent of the protein of the ration must consist of lysine in order to promote normal growth in the rat. Osborne and Mendel (54) also demonstrated the necessity of lysine for the growth of chickens.

In view of the essential role which the basic amino acids play in nutrition as brought out above, it is reasonable to assume from a survey of the analytical results of cottonseed meal secured in this investigation that the proteins of this feedingstuff have a high nutritive value. The combined arginine and histidine content of cottonseed meal is greater than that of any other feedingstuff so far analyzed with the exception of the peanut. This feature alone is of great importance in view of the fact that arginine and his-

tidine seem to be interchangeable in nutrition. While the lysine content cannot be said to be exceptional in any particular, it seems apparent from the above discussion, that the combined proteins of cottonseed meal contain sufficient amounts of both cystine and lysine to render them adequate for nutrition.

COMPARISON WITH PREVIOUS ANALYSES OF COTTONSEED MEAL. As shown in the introduction, there are but few determinations of the chemical composition of the proteins of cottonseed meal. The earliest studies were made upon one of the isolated proteins, the globulin or "edestin" of cottonseed, the results of which can not well be compared to analyses of the combined proteins, since, as previously mentioned, the globulin contains but 42.3 per cent of the total nitrogen of cottonseed meal. In the accompanying Table 4, the values secured by two previous investigators who made analyses of the combined proteins of cottonseed meal are brought together for comparison with those obtained in this investigation.

It is evident from the results presented that there is a general agreement between the three sets of values, but that there are considerable differences in several important particulars. As pointed out in the introduction, Nollau (10) calculated his results upon the total nitrogen content of the hydrolyzed solution after filtering off the solid residue insoluble in hydrochloric acid. This means that all of his calculations are too high, since a part of the nitrogen of the sample was undoubtedly discarded in the solid residue. The value of 6.27 per cent humin nitrogen reported by Nollau must, therefore, represent the soluble humin nitrogen, which is nearly as large a value as that obtained by the writer for the sum of the insoluble humin nitrogen plus the soluble humin nitrogen. The amount of soluble humin nitrogen found by the writer was but 3.89 per cent. Compared to the total humin nitrogen found by Grindley, et al (9), the amount of total humin

TABLE 4.- DISTRIBUTION OF NITROGEN IN COTTONSEED MEAL AS DETERMINED BY DIFFERENT INVESTIGATORS

(Results expressed in percentage of the total nitrogen of the feedingstuff)

Investigator	Humin N	Ammonia N	Arginnine N	Cystine N	Histidine N	Lysine N	Amino N in filtrate from bases	Non-amino N in filtrate from bases	Total N accounted for
Nollau	6.27	14,06	12.77	2.74	7.57	1.94	45.02	7.49	97.48
Grindley	7.78	10.45	19.52	0.65	5.47	4.78	42.82	5.43	96.90
Nevens	6.58	9.49	18.74	0.91	7.40	3.81	40.12	2.68	98.75[1]

[1] Includes 9.03 per cent N removed in preliminary extractions plus uncharacterized nitrogen lost in method of analysis.

nitrogen as determined by the writer was 1.19 per cent less. The reduction of the humin nitrogen has no doubt been an important contributing factor in the present investigation in securing somewhat higher values of the basic amino acids. In view of the known effects of acid hydrolysis of the proteins in the presence of carbohydrates, as already pointed out, it is reasonable to assume that the smaller amount of nitrogen discarded in the form of humin in these experiments than in those of Grindley may be attributed to the more complete separation of the proteins from the carbohydrates before hydrolysis.

The method of analysis of the proteins after hydrolysis by hydrochloric acid, as employed by Grindley et al, was similar to that employed by the writer, the main point of difference between the complete procedures being in the omission by the former workers of the extractions previous to hydrolysis. At just what point the 6.106 per cent of nonprotein nitrogen removed by the writer in the preliminary extractions might appear were it not so removed, is not clear. However, the sum of the ammonia nitrogen, amino nitrogen and non-amino nitrogen in the filtrate from the bases, obtained by Grindley et al, is 6.414 per cent greater than the sum of the corresponding values obtained by the writer, so it is possible that these three forms of nitrogen as reported by the former comprise some nitrogen not derived from the proteins as such.

It is evident from the table that the nitrogen of the bases as found by Grindley and his coworkers are in much closer agreement with those obtained by the writer than those reported by Nollau. The latter's figures for arginine are obviously too low, while his cystine values are more than four times as great as those of Grindley et al and three times as great as those of the writer. Accordingly, the lysine nitrogen values as calculated by Nollau are correspondingly too low. The values for the total nitrogen of

the bases as found by the three investigators in the order given in the table are as follows: 25.02 per cent, 30.42 per cent and 30.84 per cent respectively, the last being nearly 0.5 per cent higher than previous determinations.

The total nitrogen accounted for in the three reports is likewise shown to be 97.86 per cent, 96.90 per cent and 98.75 per cent. The greater amount in the last case is evidently due in part at least, to the inclusion of the determinations of the uncharacterized nitrogen lost at points where unavoidable losses occur in the method of analysis. These losses were not determined by the first two investigators.

COMPARISON OF THE DISTRIBUTION OF NITROGEN IN COTTONSEED MEAL WITH THAT IN OTHER FEEDINGSTUFFS. A comparison of the results of analysis of the proteins of cottonseed meal, as discussed above, with those obtained by Hamilton, Grindley and Nevens (33) for alfalfa hay, oats and corn is of value in studying the relative nutritive value of the proteins of these feedingstuffs, as well as the applicability of the general method of analysis to feedingstuffs which vary widely in composition. In the analysis of oats and corn an additional preliminary extraction, which involves the use of hot trichloracetic acid, is employed to remove the starch. This extraction is not necessary in the case of cottonseed meal and alfalfa hay on account of the absence of starch in the former, as stated by Withers and Fraps (55), and the relatively small amount of starch in the latter.

The first point of interest in contrasting these feedingstuffs, as may be noted by reference to Table 5, is their content of nonprotein nitrogen. Oats contain more than twice as much nonprotein nitrogen as cottonseed meal, while alfalfa hay contains more than three times as much. Hart and Bentley (56) found that 23.5 per cent of the nitrogen of alfalfa hay is present in a water soluble form while Grindley and Eckstein (38) found a value of 28.4

TABLE 5.- COMPARISON OF THE DISTRIBUTION OF NITROGEN IN COTTONSEED MEAL WITH THAT IN OTHER FEEDINGSTUFFS
(Results expressed in percentage of total nitrogen of the feedingstuff)

Feeding-stuff	Nonprotein Nitrogen				Results of Van Slyke Analysis										Nitrogen lost in method of analysis							Total
	Soluble in absolute ether	Soluble in absolute alcohol	In filtrate from colloidal iron	Total nonprotein nitrogen	Insoluble humin nitrogen	Soluble humin nitrogen	Ammonia nitrogen	Arginine nitrogen	Cystine nitrogen	Histidine nitrogen	Lysine nitrogen	Amino acid N in filtrate from bases	Non-amino acid N in filtrate from bases	Total nonprotein + results of Van Slyke analysis	N in residue after treatment with strong NaOH	In alcohol ppt. of hot 2 pct. CCl_3COOH extract	Unadsorbed humin (filtered from sol. during decomp. of bases.)	Soluble in amyl alcohol-ether mixture	In residue filtered from solution of bases	In residue filtered from solution of filtrate from bases	Total nitrogen lost	Total nitrogen accounted for
Alfalfa hay	0.550	1.848	16.692	19.090	3.690	4.481	7.364	7.996	0.991	3.931	4.434	38.032	2.511	92.520	2.519	-----	1.161	0.611	-----	0.441	4.732	97.252
Oats	0.569	1.225	11.129	12.926	3.013	2.516	11.422	11.647	0.944	5.795	2.841	42.137	3.860	97.100	0.132	0.127	0.664	0.746	0.209	0.025	1.903	99.004
Corn	0.326	1.368	8.135	9.829	1.235	2.303	11.936	8,725	1.072	4.832	2.200	46.704	7.216	96.052	0,136	0.276	2.698	0.481	0.191	0.065	3.847	99.899
Cotton-seed meal	0.125	0.545	5.436	6.106	2.699	3.890	9.485	18.736	0.906	7.397	3.807	40.124	2.677	95.827	0.492	-----	1.252	0.875	0.228	0.076	2.923	98.750

per cent for the same feedingstuff.

The amount of total humin is greatest in the case of alfalfa, a natural result, since the proteins are more difficultly extracted from those feedingstuffs containing large amounts of crude fiber. The amount of humin in the case of corn is very small indeed, considering the high percentage of carbohydrates in this cereal, and compares very favorably with the amounts of humin resulting from the hydrolysis of pure proteins as shown in Table 3. Cottonseed meal occupies a medium position in respect to the proportion of humin nitrogen.

The most striking difference between these four feedingstuffs is in their basic amino nitrogen content. Cottonseed meal, as already indicated, is exceptionally high in arginine nitrogen, but it is also much higher in its total basic nitrogen content than the other three feedingstuffs, the values for the four feedingstuffs being: alfalfa hay, 17.412 per cent; oats, 21.228 per cent; corn, 17.529 per cent; and cottonseed meal, 30.846 per cent. The sum of the arginine nitrogen and histidine nitrogen is more than twice as great in the cass of cottonseed meal as in the case of alfalfa hay and nearly twice as great as that of corn. From the considerations presented above regarding the biological significance of the basic amino acids, it would be logical to assume that these wide differences in the chemical composition of the proteins of different feedingstuffs indicate similar differences in their nutritive value, though probably not in corresponding degree. This point is mentioned below in connection with the discussion of the results of the feeding experiment conducted for the purpose of studying the nutritive value of the proteins of cottonseed meal.

Alfalfa hay contains the smallest proportion of mono-amino acid nitrogen, possibly owing to its high content of nonprotein nitrogen, while corn is ex-

ceptionally high in its content of both mono-amino and non-amino acid nitrogen.

The largest amount of nitrogen lost in the method of analysis occurs in the case of alfalfa, which is accounted for largely in the nitrogen remaining in the residues after the preliminary extractions have been completed. The next largest amount is in the case of corn, where the bulk of the loss is due to unadsorbed humin. The nitrogen is extracted very completely from both oats and corn. Cottonseed meal occupies a medium position with respect to the nitrogen lost in the analytical procedure.

The total nitrogen accounted for in the case of the various feedingstuffs is a point worthy of special note. The total is least in the case of alfalfa and greatest with corn. Here again cottonseed meal occupies a medium position. In this rather long method of analysis, which involves many extractions, concentrations, precipitations, filtrations and transfers, and which at some stages renders the proteins subject to putrefaction unless care is taken, only 0.101 per cent of the total nitrogen originally present in the sample of corn was not accounted for, a very remarkable result indeed.

An examination of the results of individual analyses of the four samples of alfalfa hay and six samples of each of oats and corn which were averaged to obtain the values shown in Table 5, brings out the fact that the analytical results in the case of each of these feedingstuffs show, on the whole, less variability than the values for the eight samples of cottonseed meal shown in Table 1. At least two factors operated to effect the difference. The analyses of the first three feedingstuffs mentioned were conducted by persons experienced in the manipulation and execution of the Van Slyke analysis and the analyses used for the averages were selected from a number of analyses. The analyses of cottonseed meal were made by the writer who had had no previous experience in the conduct of the Van Slyke method, and

the analyses presented in Table 1 are the entire results of the work. These considerations are strong evidence that the method of analysis here described is of general application to feedingstuffs and may readily be carried out.

SUMMARY OF THE DISCUSSION OF THE RESULTS OF THE CHEMICAL ANALYSIS OF THE PROTEINS. The accuracy of the determination of the amino acid content of the proteins of cottonseed meal has been increased over that of previous methods by the removal of the nonprotein nitrogen before proceeding with the hydrolysis of the proteins.

The accuracy of the determination has been still further increased by the reduction of the humin substances formed as a result of the hydrolysis of the proteins.

The amount of arginine nitrogen is much higher than that in most other feedingstuffs. The sum of the four basic amino acids is about 0.5 per cent higher than values previously found for cottonseed meal.

The method of extraction employed was found to result in the removal of 99.5 per cent of the total nitrogen present in the feedingstuff.

The sum of the ammonia nitrogen and amino acid nitrogen fractions is 83.132 per cent of the total nitrogen, an amount comparable to the sum of the same fractions previously obtained from pure vegetable proteins.

Of the total nitrogen originally present in the sample of cottonseed meal, 98.75 per cent was accounted for by summation of the fractions obtained at different stages in the method of analysis, a proportion greater than any previously reported for the same feedingstuff.

Judging from the results obtained in this investigation, the complete method of analysis outlined in this paper is probably of general application to feedingstuffs and may readily be executed with successful results.

IV. METHODS EMPLOYED IN STUDYING THE NUTRITIVE VALUE OF THE PROTEINS OF COTTONSEED MEAL

OBJECT OF FEEDING EXPERIMENT. The object of this phase of the experiment was to study the nutritive value of the proteins of cottonseed meal and to compare their nutritive value with that of the proteins of corn and alfalfa hay for the growth of young albino rats. It was planned to feed rations containing a medium amount of protein, derived from the above mentioned sources, and by means of metabolism studies to determine the extent to which the proteins are utilized for maintenance and growth.

GENERAL PLAN OF EXPERIMENT. Young male albino rats in vigorous, healthy condition and having an initial weight of from 100 to 140 grams were employed. The metabolism periods were each 7 days in length, two such periods following each other without intermission with each of the experimental rations tested. Before the first metabolism period and whenever the rations were changed, a three day preliminary or transition period during which the ration to be employed during the metabolism period was fed was inserted. It was planned to feed tha animals as large amounts of the rations as they would consume, the daily feed allotment being slightly greater than the amount consumed.

The rats were placed in individual glass crystallizing dishes $7\frac{1}{4}$ inches in diameter and $3\frac{3}{4}$ inches in depth, inside measurements. The dishes were provided with weighted wire covers to which were attached large test tubes fitted with rubber stoppers and bent glass tubing, the latter extending downward thru the wire cover. The test tubes were kept supplied with ammonia-free water. Large porcelain crucibles for receiving the feed were supported from the covers by means of wire frames. Crystallizing dishes of 60 mm. diameter were employed instead of the crucibles for rations containing alfalfa, which were very bulky. Ventilation was provided by means of a system of rubber tubes which conducted a current of compressed air to the bottom of each

dish. From two to three sheets of filter paper, cut to fit the dishes, was placed in the bottom of each dish daily to absorb the urine.

Feces and urine were collected daily. In most cases the filter paper absorbed the urine completely, so that the feces were nearly always found dry. In a very few cases, particularly with rations containing alfalfa which resulted in the production of very bulky feces, there was evidently absorption of urine by the feces, so that it was necessary to extract the feces once or twice with hot acidified water before collecting them. The feces were preserved under 95 per cent alcohol acidified slightly with sulfuric acid. At the end of each 7 day metabolism period, the feces were transferred to large Kjeldahl flasks and digested according to the Kjeldahl-Gunning-Arnold method with sulfuric acid, sodium sulfate and mercury. The resulting solutions were transferred to 500 c.c. volumetric flasks and aliquots taken for distillation.

After collecting the feces, the urine was extracted from the filter papers by washing with a stream of ammonia free water acidified with sulfuric acid and held at nearly boiling temperature. The filter paper was thoroly pulped and pressed out after each extraction by means of a glass rod. From four to six extractions were made, using 40 to 60 c.c. of water each time, the sides and bottom of the dish also being thoroly washed. The extracts were filtered thru glass wool into 250 c.c. volumetric flasks. The flasks were allowed to remain in the ice box over night. The solutions were then made up to volume at ice box temperature and transferred to 2 $\frac{1}{2}$ liter bottles which were kept in a cold storage room at a temperature of 5 degrees to 10 degrees C until analyzed. About one half gram of powdered thymol was employed as a preservative in each bottle in which the week's urine was collected. The composites were thoroly mixed and aliquots measured out in the cold for total nitrogen determinations.

The feed was weighed daily into the crucibles and mixed with a little nitrogen free water to the consistency of a thick paste. The following day the

feed residues were scraped out and dried in the same oven and at the same temperature as the rations used. In some cases the animals scattered the feed from the crucibles about the metabolism dish. In such cases the feed remaining in the metabolism dish at the time of collecting the excreta was carefully separated and added to the feed residues. When thoroly dry the weight of the feed residues was determined and the amount of feed actually consumed during the 7 day period calculated. Byprevious tests in this laboratory it was found that the error involved in this calculation due to a difference in the moisture content of the residues and ration was less than 1 per cent, and further that the nitrogen contents of the residues and ration were identical (57).

PREPARATION OF RATIONS. In preparing the experimental rations, the starch used was first dextrinized by heating on the steam bath after the addition of cold water and a few crystals of citric acid. When ground corn formed one of the constituents of a ration, it was mixed with the starch and the starch of the mixture dextrinized. The other ingredients were then added, the agar being dissolved in boiling water and added at the boiling temperature. When necessary more hot water was added and the ingredients thoroly mixed. The rations were dried on glass plates, placed above the steam bath, finely ground and dried in an oven at a temperature of about 40 degrees C. After drying for several days, the rations were mixed, sampled for analysis and placed in tightly covered glass jars.

The nitrogen free ration consisted of the following;

Salts	5	per cent
Butterfat	10	" "
Sucrose	8	" "
Starch	74	" "
Agar	3	" "

Water soluble vitamin, 150 mg. of solids per 100 grams of ration.

The composition of the other rations is shown in Table 6. The salt mixture used was compounded according to the formula of Osborne and Mendel (58), while

TABLE 6.- COMPOSITION OF EXPERIMENTAL RATIONS

(Expressed in percentage)

Constituent	Ration						
	1[1]	2	3	4[1]	5	6	7
Cottonseed meal	23.9	----	----	13.1	----	10.3	7.7
Corn	----	72.7	----	32.3	28.4	----	19.3
Alfalfa hay	----	----	63.5	----	40.6	35.7	29.3
Starch	55.1	6.3	18.5	33.7	13.0	36.0	25.7
Agar	3.0	3.0	----	3.0	----	----	----
Sucrose	5.0	5.0	5.0	5.0	5.0	5.0	5.0
Butterfat	10.0	10.0	10.0	10.0	10.0	10.0	10.0
Salts	3.0	3.0	3.0	3.0	3.0	3.0	3.0
Total	100.0	100.0	100.0	100.0	100.0	100.0	100.0
Total nitrogen content	1.750	1.660	1.806	1.777	1.708	1.790	1.782

[1] Water soluble vitamin preparation added at the rate of 1.5 mg. of solids per gram of ration to rations 1 and 4.

the water soluble vitamin consisted of Osborne and Wakeman's (59) Fraction II of the concentrated extract of the water soluble vitamin of brewers' yeast. The stock supply of the latter was prepared in the form of a water solution which was preserved by means of a small quantity of chloroform and kept in the ice box. The butterfat was obtained by placing fresh creamery butter in large beakers, heating to a temperature of 50 degrees to 60 degrees on the steam bath, centrifuging for an hour or until the fat became water clear, and then siphoning off the clear fat.

It was planned that all rations containing a protein feedingstuff should carry 10 per cent of protein (N x 6.25), but the actual content of protein was slightly higher, ranging from 10.38 per cent to 11.28 per cent, due to the fact that some of the constituents used in making up the ration had a slightly higher moisture content than the dried rations.

In preparing the rations in which two or more feedingstuffs were combined an effort was made to have each feedingstuff furnish an equal amount of digestible protein, using the coefficients of digestibility secured in Period 2 as a basis for calculation, but keeping the total content of crude protein the same thruout the experiment, namely, 10 per cent.

The cottonseed meal used in the rations was a part of the same sample which was employed in the analytical study presented in the preceding section. Thru the courtesy of the Plant Breeding Division of the Agronomy Department of this university a quantity of "high protein" corn containing 2.2 per cent nitrogen was secured, which made it possible to formulate a suitable corn ration containing 10 per cent protein. All of the feedingstuffs used were in a finely ground condition before compounding the rations.

ACCURACY OF METABOLISM WORK WITH SMALL ANIMALS. Since the accuracy of metabolism work depends in large measure upon the accuracy of the collection of the excreta, especially when such small amounts of nitrogen are involved as in

the case of the smaller laboratory animals, several experiments were carried out to test the accuracy of the methods employed.

Each day during Period 6, when 6 rats were receiving the same feed mixture, the paper residues remaining after extraction of the urine were collected in glass jars and placed in the ice box. At the end of the metabolism period, the entire mass of residues, including the glass wool used in filtration, was transferred to a two liter beaker and boiled for some time with about one liter of water acidified with sulphuric acid. The extracts were decanted and the procedure repeated, the acidified water being pressed out from the residues. The extracts were filtered thru glass wool, evaporated on the steam bath and transferred to Kejldahl flasks for total nitrogen determination. The paper residues also, together with the glass wool, were transferred to large Kjeldahl flasks, and total nitrogen determined. The results of these determinations are shown in Table 7. The nitrogen extracted in the procedure described just above is assumed to be of urinary origin and is compared to the total amount of urinary nitrogen excreted during the week. It is shown that, as an average of 6 such determinations, the error in the collection of urine amounted to 2.0 per cent of the total nitrogen.

The nitrogen remaining in the paper residues which was not extracted by boiling with acidified water was assumed to be fecal nitrogen. In collecting the feces it was sometimes impossible to entirely remove the fecal matter from the filter papers, especially when the rations tended to cause a laxative condition. Such a condition was not a constant effect with any ration employed, but was more frequent with rations containing alfalfa. With the latter rations, three filter papers were generally placed in each dish daily, while with the other rations two papers were used. Analysis of the filter paper showed that seven filter papers of the size used contained 1.06 mg. of nitrogen. In making

TABLE 7.- TEST OF THE COMPLETENESS OF EXTRACTION OF NITROGEN FROM FILTER PAPERS USED AS ABSORBENTS DURING ONE METABOLISM PERIOD

Rat No.	Extractable N in paper pulp	N in urine collected	Total urinary N excreted	Error in collection of urine	Non-extractable N in paper pulp[1]	N in feces collected	Total fecal N excreted	Error in collection of feces	Total N in paper pulp	Total N of excreta for period	Total error in collection
	mg.	mg.	mg.	pct.	mg.	mg.	mg.	pct.	mg.	mg.	pct.
2	12.5	558.4	570.9	2.2	25.5	546.8	572.3	4.5	38.0	1143.2	3.3
3	15.9	716.3	732.2	2.2	25.8	833.7	859.5	3.0	41.7	1591.7	2.6
5	9.0	619.3	628.3	1.4	22.2	645.3	667.5	3.3	29.2	1295.8	2.3
6	13.2	541.0	554.2	2.4	20.1	615.8	635.9	3.2	33.3	1190.1	2.8
8	11.5	652.5	664.0	1.7	12.9	672.0	684.9	1.9	24.4	1348.9	1.8
9	18.3	764.3	782.6	2.3	28.0	602.0	630.0	4.4	46.3	1412.6	3.3
Aver.	13.4	-----	655.4	2.0	22.4	-----	675.0	3.3	35.5	1330.4	2.7

[1] After deduction of nitrogen contained in same number of clean filter papers.

the calculations shown in Table 7 it was assumed that the nitrogen originally contained in the filter papers was insoluble in the hot dilute acid employed in extraction and this has been deducted from the non-extractable nitrogen remaining in the paper residues. To the extent that such nitrogen is soluble in hot dilute acid, however, it would tend to offset to a small degree the losses in the collection of urine, altho the correction would not be in proportion to the variable total urinary nitrogen. The error in the collection of feces was found to be 3.3 per cent, using the average of six determinations, or, comparing the total nitrogen lost to the total nitrogen excreted in urine and feces during the week, the total error in the collection of both feces and urine is 2.7 per cent.

The results obtained in this test were applied to the metabolism data for Period 6, the period during which this test was conducted, to determine what effect the incomplete collection of the excreta has upon the utilization coefficients. It is evident that an error in the collection of the excreta is reflected directly in the nitrogen balance and in the percentage utilization. Using the average values given in Table 7 of 13.4 mg. of nitrogen representing uncollected urine and 22.4 mg. of nitrogen representing uncollected feces during a 7 day period, and applying them to the data for the individual animals during Period 6 it is found that the percentages of utilization of absorbed nitrogen as given in the tables are approximately 2 per cent too high, while the percentages of absorbed nitrogen retained are slightly more than three per cent too high. Like results are obtained for Period 7 during which the animals received the same ration as in Period 6.

The completeness of the collection of urine was tested in still another way, that of the recovery of urea which was added in the form of a standard solution to the daily feed. Two mature rats were employed in the test. After the excretion of urinary nitrogen had been reduced to a nearly constant level by subsistence on protein free rations for seven days, known amounts of urea were added

to the ration.

The excretion of this extra nitrogen was very prompt, as indicated by the results of the test as shown in Table 8. In the case of Rat 10, the results are somewhat difficult to interpret, owing to the fact that the consumption of feed decreased rapidly, evidently resulting in catabolism of small amounts of body protein to furnish energy for the body. By using the figures for the average excretion of nitrogen during the three days preliminary to the first urea day as the level of the endogenous nitrogen during the three urea days, the apparent recovery of the urea nitrogen amounted to 119 per cent. Similar results are obtained if the average nitrogen excretion during the preliminary and subsequent periods are employed. If it be assumed that, owing to a decrease in feed consumption below that of the energy requirements, the endogenous nitrogen should be taken as corresponding to that in the subsequent period, then the recovery of the urea nitrogen was approximately 100 per cent. Undue enphasis should not be placed upon the results given by this animal, however.

With Rat 11 more reliable data were obtained as it was not evident that the feed consumption was deficient in meeting the energy requirements. By using the average nitrogen excretion during both preliminary and subsequent periods as the level of the amount of body nitrogen excreted, the recovery of urea nitrogen amounted to 95 per cent of that fed. If the average of the amounts of nitrogen excreted during Days 3 and 6 be used as this level, then the recovery of urea nitrogen was practically 100 per cent.

It is evident from the data presented concerning the recovery of urea nitrogen that the method for the collection of urine as employed in these experiments gives very nearly quantitative results.

Further tests of the metabolism method employed which were performed in this laboratory and are described below, show that loss of ammonia due to bacterial decomposition of the urine does not occur to any appreciable extent.

TABLE 8.- TEST OF THE COMPLETENESS OF COLLECTION OF URINE BY ADDITION OF UREA TO THE RATION

Day	Live weight	Feed eaten	Urea N added	Daily urinary N
	gm.	gm.	mg.	mg.
		Rat 10		
1	182	10.5	0	35.6
2	---	10.5	0	24.0
3	---	10.5	0	23.5
4	178	6.1	42.1	74.6
5	---	6.1	42.1	83.0
6	---	6.1	42.1	76.0
7	---	4.6	0	35.9
8	167	4.6	0	35.8
		Rat 11		
1	176	7.2	0	42.8
2	----	7.2	0	30.2
3	----	7.2	0	39.8
4	----	7.7	56.5	82.8
5	----	7.7	56.5	101.8
6	----	5.4	0	37.5
7	164	5.4	0	29.3

In the first test, three portions of urine of 5 c.c. each were measured out for total nitrogen determination. At the same time 5 c.c. portions of urine were added to each of six metabolism dishes containing the usual number of filterpapers. Three of these dishes were allowed to stand at room temperature in the metabolism laboratory, while the remaining three were placed in an oven at a temperature of about 40 degrees C. At the end of 24 hours, the urine was collected from all six dishes in the same manner as employed in the metabolism work, i.e., by washing with hot, acidified water. As a result of this test it was found that 5 c.c. of urine contained 28.18 mg. of nitrogen, while the amounts of nitrogen recovered from the dishes kept for 24 hours at room temperature and at 40 degrees C were, respectively, 27.61 mg. and 27.54 mg.

In the second of these tests, the urine from one rat receiving a constant amount of the same ration was collected daily. On the first, third, and fifth days the urine was collected at once by washing with acidified water in the usual manner. The urine was made up to a volume of 250 c.c. and aliquots taken at once for total nitrogen determinations. On the alternate days the urine was not collected at the end of the 24 hour period, but the filter paper was moistened and the dish allowed to stand another 24 hours in the metabolism laboratory before extraction in the usual manner. The rat meanwhile was transferred to a clean dish. At the end of the second day the urine was collected by washing as usual, made up to volume, and aliquots taken for total nitrogen determination. The results of the test are shown in Table 9. It is evident that there was no appreciable loss of nitrogen due to bacterial decomposition even after the metabolism dishes had stood for two days.

HOW SHALL THE NUTRITIVE VALUE OF PROTEINS BE COMPARED? In attempting to compare the biological values of various feedingstuffs, it is first necessary to select a suitable basis for comparison. Several different methods for comparison are in use. As pointed out in the introduction, one of the most common methods

TABLE 9.- EFFECT OF ALLOWING METABOLISM DISHES TO STAND 24 HOURS AND 48 HOURS BEFORE THE COLLECTION OF URINE

Day of experiment	Daily urinary N when collected at end of 24 hours	Daily urinary N when collected at end of 48 hrs.
	mg.	mg.
1	57.9	----
2	----	64.9
3	60.8	----
4	----	67.3
5	63.2	----
6	----	60.3
Average	60.3	62.1

is to base conclusions upon the character of the growth secured, the principle index in such a case being the gain in live weight. Some of the data from Table 22 of the appendix are brought together in Table 10. These data were all obtained in Periods 2 and 3. The rats consuming the cottonseed meal ration showed marked fluctuations in gain in live weight which can not be accounted for on the basis of a variable food intake. With the corn ration, there was a gain in weight by one rat in one period only. The nitrogen of the ration, however, was being used by the body to a considerable extent, for on a nitrogen free ration the same animals lost 16 to 19 grams in weight during a period of equal length, compared to 1 to 2 grams on the corn ration. Likewise, there was also a large variation in gains in weight by the rats receiving the alfalfa ration, the average gains of Rats 7 and 8 being almost zero. It is probable that many factors other than the quality and amount of the protein consumed influence the gain in weight, such as the proportion of carbohydrates in the ration, amount of water drank, exercise, the proportion of gain which is protein or fat, etc. While interpretations based upon the gain in live weight may lead to reliable conclusions in some instances, the adoption of such a criterion in the present case would certainly be a fallacious procedure.

It is a matter of common knowledge that all animals require protein food for keeping the body tissues intact, known as the maintenance requirement, and secondly, that growing animals need an additional quantity of protein for the construction of new tissues. If a standard for the comparison of the value of the proteins of feedingstuffs for growth is based simply upon the proportion of the nitrogen of the feedingstuff which is retained by the body, the values secured in such a manner are subject to gross errors. With such a method of computation the apparent value of the proteins for growth depends largely upon the nitrogen intake, or in other words, upon the amount of feed eaten, and this in turn is subject to individual idiosyncracy and the palatability of the ration.

TABLE 10.- A COMPARISON OF THREE METHODS OF EXPRESSING THE UTILIZATION OF PROTEINS

Rat No.	Period	Ration	Feed consumed daily	Gain in weight for period	Absorbed N retained[1]	Utilization of absorbed N[1]
			gm.	gm.	pct.	pct.
1	2	cottonseed meal	9.52	11	31	63
1	3	"	9.48	6	29	65
2	2	"	8.66	9	21	64
2	3	"	8.57	5	17	64
3	2	"	11.51	12	44	71
3	3	"	12.47	11	43	70
4	2	corn	7.44	2	15	49
4	3	"	8.05	0	15	47
5	2	"	7.66	0	16	54
5	3	"	6.30	-1	--	43
6	2	"	7.49	-1	23	55
6	3	"	6.35	-2	9	48
7	2	alfalfa hay	9.33	6	23	62
7	3	"	9.50	-5	8	57
8	2	"	9.19	2	16	58
8	3	"	8.20	-2	6	57
9	2	"	11.42	3	22	67
9	3	"	13.67	7	38	73

[1] For method of calculation of these percentages, see table 22 of the appendix

As mentioned elsewhere, when the ration proves unsatisfactory, rats tend to eat less and less from day to day. By reference to Table 10 it may be seen that both Rats 5 and 6 ate less of the corn ration during Period 3 than during Period 2. Rat 5, during Period 2, retained 16 per cent of the nitrogen absorbed, but during Period 3 when the amount of feed consumed was evidently too little to maintain the animal's live weight, the nitrogen of the excreta was greater than the nitrogen intake, so that there was a loss of nitrogen from the body resulting in a negative value for the percentage of absorbed nitrogen retained. Similarly, the percentage of absorbed nitrogen retained by Rat 6 falls from 23 per cent in Period 2 to 9 per cent in Period 3, a change which in this instance may also be attributed to a decreased food intake. Were the average percentage of the absorbed nitrogen retained by Rats 5 and 6 taken as a measure of the utilization of the proteins of corn for growth, it would be a distorted picture of the facts.

There seem to be factors other than the amount of feed consumed which render the use of the percentage of absorbed nitrogen retained an unsatisfactory criterion of the utilization value of the proteins of feedingstuffs. As may be seen by reference to Table 10, the percentage of nitrogen retained by Rat 7 in Periods 2 and 3 falls from 23 per cent to 8 per cent, and in the case of Rat 8 the percentage falls from 16 per cent in Period 2 to 6 per cent in Period 3. These violent flucutuations are not due entirely to a decreased nitrogen intake, for in the case of Rat 7 the feed intake increased slightly during the second period. They are, however, associated with a slightly decreased digestibility, altho there may be other causative factors.

Any suitable criterion used in feeding experiments for the comparison of the utilization of proteins for growing animals must necessarily consider the effect of the proteins in providing nitrogen for maintenance, for in growing animals these processes proceed concurrently. It is doubtful if the true protein requirement for the maintenance of a growing animal can be determined by feeding a ra-

tion containing protein, for Waters (60) has shown that when young steers received a ration which just maintained their live weight, some of the growth processes continued. Similar results were obtained by Aron (61).

Perhaps the nearest approach to the determination of the exact amount of nitrogen required for the maintenance of a growing animal is a study of the nitrogen excretion when the ration consists entirely of carbohydrates and this is being taken in an amount in excess of the body's energy requirement. Under such conditions the nitrogen excretion falls to a very low level, often to one third or less of that during starvation, as shown by Folin (62), Landergren (63), Cathcart (64), and Thomas (65). The amount of protein then being catabolized has been defined by Rubner (66) as the "wear and tear" quota of protein metabolism, which requires a "repair quota" of protein in the diet in order to replace it. A "growth quota" must be supplied the young animal in addition to the "repair quota" in order that growth may take place. Using dogs as experimental animals, Michaud (67) found that when protein in the form of casein or dog tissue was fed in amounts equivalent to the protein minimum after the metabolism had been reduced to this level, that there was no further loss of nitrogen from the body. Thomas (65) found that after the reduction of the nitrogen excretion by a carbohydrate diet to the minimum level that nitrogen equilibrium could be restored by the ingestion of an amount of protein nitrogen in the diet equal to the amount of nitrogen being eliminated in the excreta. On a nitrogen free diet the amount of nitrogen excreted daily in the feces was about 1 gram, and this amount was not increased with a nitrogen intake of 3 grams furnished by a highly digestible protein. With diets producing a large bulk of feces he found that a greater proportion of digestive juices was eliminated, increasing the nitrogen content of the feces.

In the interpretation of the feeding experiments which follow it is assumed that the amount of protein required for body maintenance is a constant value

for each individual at a given weight. Such an assumption is entirely in harmony with the theories of many investigators in the fields of both human and animal nutrition. Folin (62), as a result of his study of the different forms in which nitrogen is excreted on high and low protein diets, was led to formulate his theory of two distinct types of metabolism. The endogenous is most characteristically represented by the excretion of creatinine, which, "on a meat free diet is a constant quantity, different for different individuals, but wholly independent of quantitative changes in the total amount of nitrogen eliminated." Folin's results have been substantiated by an immense amount of investigation concerning urinary creatinine, and his theory of protein metabolism is now almost universally accepted in its main essentials, altho it has been necessary to modify this view slightly with our increased knowledge of the chemistry of the proteins.

The constancy of the protein minimum for the individual is accepted by Lusk, Thomas and others. That this minimum differs between individuals and is subject to slight variation due to environmental, temperamental and dietary changes, possibilities which are not precluded by Folin's theory, is brought out by Cathcart (68). "As regards the uniformity of the protein minimum it may be definitely stated that there is no single minimum - common to all men and to all conditions. Rubner, Caspari and others also hold firmly to this opinion. Caspari quotes the work of Larguier des Bancels in 1903 in confirmation of this belief in the existence of multiple protein minima. The facts that can be cited against a common minimum are many in number. Thus the caloric value of the diet given influences very materially the amount of nitrogenous material required, as is shown, for example, in the experiments of Voit and Korkunoff. Then, as Rubner has pointed out, the temperature influences quite markedly the course of protein metabolism. Finally, another factor of considerable importance may be mentioned, the activity of the organism."

In the sphere of animal nutrition, the constancy of the maintenance re-

quirement for farm animals is recognized by Kellner, Armsby and Haecker. C. Voit and Kellner also proved conclusively that work production of varying intensity by farm animals does not increase the protein metabolism appreciably.

In this connection it should be stated that some of the current theories of protein metabolism are not in complete harmony with that just mentioned. Among these are the reversible reaction theroy of Sherman (69), which seeks to account for the functions which the food protein serves in body maintenance by the assumption that the absorption of the amino acids liberated in digestion causes an increased concentration of these in the tissues which checks or even reverses the hydrolysis of tissue protein. This theory is hardly compatible with the known facts regarding the constancy of the endogenous metabolism, which has been found (70) to be uniform from hour to hour, as evidenced by the creatinine elimination, even during digestion and absorption of proteins. Absorption of the protein digestion products from the alimentary tract presumably occupies only a portion of the 24 hour period, so that even if the endogenous catabolism were inhibited by the increased concentration of amino acids, it would be only temporary, for it has been shown (71) that, in adult rats, protein feeding has only a very slight effect upon the amino acid concentration in the tissues. This known slight increase in the amino acid content of the tissues during digestion would not be of sufficient magnitude to inhibit the action of digestive enzymes when a digestion experiment is conducted in vitro. Further, it is unreasonable to assume that anabolism and catabolism of tissue proteins are simply reversible phases of the same reaction and that both these processes are promoted by the same enzyme. In the young growing animal protein feeding has been demonstrated (71) to increase considerably the amino acid content in the tissues. Were the endogenous metabolism inhibited entirely during the time this concentration is maintained, as must be assumed from the reversible reaction theory, then the catabolism of tissue protein per unit of weight in the young growing animal would be but a

fraction of that of a mature animal.

Osborne and Mendel (72) explain the maintenance protein requirement upon the need of certain amino acids to serve special physiological functions, such as the formation of the active principles of the internal secretions and hormones. This theory assumes, therefore, that when the animal is receiving a nitrogen free ration, body tissue must be catabolized to furnish the essential amino acids, but that, on the other hand, when a ration containing a complete assortment of amino acids in sufficient amount is being consumed that the endogenous metabolism is only a fraction of that on a non-nitrogenous ration, and that then only the catabolism of the internal secretions or the tissues which regulate metabolism would be effected. Under these conditions the muscles would scarcely be affected and the creatinine elimination would bear little relation to the endogenous metabolism. Moreover, the theory does not satisfactorily account for the effect of ammonium salts, mixtures of amino acids and single amino acids in partially supplying nitrogen for maintenance.

Since the plan of procedure and method of calculation employed in this investigation are dependent primarily upon the basic assumption that the endogenous metabolism of the animal organism is constant in character and amount for an individual at a given age and weight, an examination of the data obtained during all the metabolism periods was made in order to ascertain, if possible, whether this assumption is substantiated by the experimental results at hand. In making this examination, the data embodied in Appendix Table 22 were employed to obtain the first set of values shown in the column headed "As determined" under each "Period" of Table 11. These values were obtained by deducting the sum of the endogenous nitrogen and the metabolic nitrogen in the feces from the daily urinary nitrogen and calculating the percentage of the daily nitrogen intake which the remainder forms. The second column of values under each "Period", headed "As calculated", was obtained in the same way as those in the first column, except that

TABLE 11. - THE PROPORTION OF THE DAILY INTAKE OF NITROGEN ABOVE MAINTENANCE WHICH APPEARS IN THE URINE
(Expressed in percentage)

Rat No.	Period 2		Period 3		Period 4		Period 5		Period 6		Period 7	
	as det'd.	as calc.	as det'd.	as calc.	as det'd.	as calc.	as det'd.	as calc.	as det'd.	as calc.	as det'd.	as calc.
2	12.0	16.3	9.8	13.1	1.7	5.3	8.3	12.4	8.9	13.4	8.8	13.7
3	8.8	8.3	9.4	8.9	7.8	7.2	7.3	6.9	9.9	9.3	9.9	9.2
5	26.5	29.1	35.0	38.2	18.7	21.0	18.5	21.1	12.3	14.4	11.4	13.7
6	23.9	20.0	31.3	26.7	21.0	17.5	16.4	12.3	12.6	9.5	14.0	11.1
8	9.1	7.4	7.8	5.8	15.6	14.3	10.4	8.9	14.3	12.5	9.7	17.7
9	4.1	5.5	1.9	3.1	15.8	17.3	14.9	16.4	16.0	17.6	16.4	18.1

that the average value for daily urinary nitrogen as determined with nine rats in period 1, i.e., 22 mg. per 100 gm. live weight, is used in calculating the "endogenous nitrogen" of Periods 2 to 7, inclusive, instead of the individual values determined in the same period. It is shown in Table 11 that the individual daily urinary nitrogen values for Rats 2, 5 and 9 are above the average, while that for Rats 3, 6 and 8 are below the average. Hence, for comparison, Rats 2 and 3, 5 and 6, and 8 and 9 are arranged in pairs. The rats of each pair received the same rations thruout the experiment. It is natural to assume that two animals of the same age and weight and in a comparable nutritive condition will utilize the same ration with an equal degree of efficiency, subject of course, to inherent individual variability. By reference to Table 11, it may be noted that this holds true to a very great extent, altho the natural variations to be expected in biological work of this kind are in evidence.

Considering first the values listed under the headings "As determined" in each period it is evident that there is a marked uniformity exhibited by the animals of each pair thruout the different experimental periods with but few exceptions. For example, it is shown that Rats 2 and 3 eliminate in the urine about the same proportion of the nitrogen intake above maintenance "As determined", with the exception of Periods 2 and 4. Rats 5 and 6 show quite uniform results thruout the entire six periods. Rats 8 and 9 do not vary widely from each other in Periods 4, 5 and 6. Moreover, certain rations seem to have pronounced effect on these percentages. Rats 5 and 6 received the corn ration during periods 2 and 3, and the corn-cottonseed meal ration during Periods 4 and 5. With both of these rations, the proportion of the daily nitrogen intake eliminated in the urine was greater than with the other rations, but both animals behaved alike in this respect.

On the other hand, when the average values for the endogenous nitrogen of the urine are used in calculating maintenance, the variations in the percentages

of the nitrogen intake above maintenance appearing in the urine of the animals of each pair are greatly exaggerated in 14 of the 18 cases involved, as shown under the headings "As calculated". In one of the four cases, that of Rats 5 and 6, P period 7, there is no change. In the other three cases, those of Rats 2 and 3, period 4, and Rats 8 and 9, periods 2 and 3, the spread is lessened slightly. In many cases the use of an average maintenance factor increases the spread between the animals of a pair as much as 200 per cent. These data seem to indicate quite conclusively that the endogenous metabolism is a function of the individual animal and that this is a definite and probably constant value under a given set of conditions. This is quite in harmony with the theory of Folin (62) respecting the constancy of endogenous metabolism. In calculating the results of these experiments, therefore, the use of individual maintenance values is evidently justifiable.

If, having determined the minimal "wear and tear" quota of an animal by appropriate metabolism experiments, feeding tests are then initiated to study the utilization of the proteins of feedingstuffs by that animal, it is possible to calculate the proportion of the nitrogen excreted in the urine which is of endogenous origin and likewise the amount of fecal nitrogen whose source is metabolic. This method follows closely that of Thomas (65) in calculating the biological values of foodstuffs. Such a criterion evaluates the proportion of the nitrogen of the food which is actually utilized by the animal in its metabolism, whether the animal is consuming an amount of protein which is not quite sufficient for it to maintain its live weight, or whether growth is permitted. The values obtained by applying this method of calculation to the data of metabolism Periods 2 and 3 are shown in the last column of Table 10. An inspection of these values shows that this method overcomes some of the objections raised to the other methods. When the gain in live weight falls to zero or a little below, but at the same time it is evident that some of the nitrogen of the ration is being used by the

animal, the percentage "utilization of the absorbed nitrogen for maintenance and growth" is lowered, but is, nevertheless, a very distinct positive value. With a slight decrease in food intake, there is usually a corresponding fall in the "utilization of absorbed nitrogen for maintenance and growth" coefficient, but this decrease is not so extreme as when the results are calculated upon the basis of the "absorbed nitrogen retained." Moreover, a decrease in the feed intake to just below the maintenance level does not result in a negative value. On the whole the "utilization of absorbed nitrogen for maintenance and growth" coefficients are much less variable than those of the "absorbed nitrogen retained". The former method is subject to a coefficient of variability of 8.4 per cent when all of the values obtained in the six metabolism periods are considered, and a mean is assumed for each ration. Similarly, the same values when calculated upon the basis of the percentage of "absorbed nitrogen retained" have a coefficient of variability of 27.7 per cent, a striking and important difference.

In this investigation, therefore, the endogenous metabolism of the experimental animals was studied during a metabolism period in which a nitrogen free ration was fed, and this was followed by six metabolism periods in which the proteins of cottonseed meal were compared with those of corn and alfalfa hay.

V. DISCUSSION OF THE RESULTS OF THE STUDY OF THE NUTRITIVE VALUE OF OF THE PROTEINS OF COTTONSEED MEAL

METABOLISM OF THE RAT ON A NITROGEN FREE RATION. During the first 11 days of the experiment the rats which had been consuming an ordinary stock ration were given nitrogen free rations prepared as described above. On the fifth day collection of the feces and urine was begun, and continued for 7 days. In order to check the results secured during the first period of the experiment, three of the rats were again placed on nitrogen free rations during Period 6, after having received nitrogenous rations during the intervening time. The principal data of these trials are included in Table 12. The rats lost slightly more than 2 grams of weight per head daily. For the first few days of the period the animals ate the ration in large quantities , but as they apparently found the feed unsatisfactory, they consumed smaller and smaller amounts from day to day, and a few of the animals scattered the feed from the containers at once upon being fed.

It was found that, while subject to some individual variation, the amount of urinary nitrogen per 100 grams of live weight is fairly constant, the average value of 22.4 mg. obtained agreeing almost exactly with that found by Mitchell (57) in a large number of metabolism periods. From the results secured in Period 6 it appears that there is slightly less intense endogenous metabolism as the animal becomes older, as evidenced by a decreased excretion of urinary nitrogen per 100 grams live weight. The slight increase in the case of Rat 7 during Period 6 as compared with Period 1 is evidently due to a deficient food consumption during the former period, necessitating the catabolism of body protein to furnish energy. The individual values obtained in Period 1 for urinary nitrogen per 100 grams live weight are used in the subsequent tables for calculating the utilization of the various rations, the values always being corrected to the average live weight of the particular animal during that period.

TABLE 12.- METABOLISM OF THE RAT WHEN RECEIVING A NITROGEN FREE RATION

Rat No.	Period	Initial weight	Final weight	Aver. feed consumed daily	Daily urinary nitrogen	Daily fecal nitrogen	Daily urinary N per 100 gms. live wt.	Fecal nitrogen per 100 gms. feed
		gm.	gm.	gm.	mg.	mg.	mg.	mg.
1	1	99	88	6.51	25	17	27	253
2	1	118	102	7.43	31	19	28	255
3	1	134	116	9.07	26	24	21	259
4	1	135	118	8.79	.24	22	19	251
5	1	120	104	7.29	28	20	25	273
6	1	129	110	7.86	21	21	18	270
7	1	123	107	7.43	23	20	20	266
8	1	122	109	8.14	22	24	19	298
9	1	126	114	7.43	29	18	25	246
Average		---	---	7.78	--	--	22	264
1	6	113	102	6.10	22	19	20	306
4	6	135	130	9.02	16	20	15	223
7	6	118	105	4.76	24	17	21	357
Average		---	---	6.63	--	--	19	295

The quantity of fecal nitrogen per 100 grams feed when the rat is consuming a nitrogen free ration is quite a constant factor, altho this relationship seems to be affected somewhat by extremes in the amount of feed consumed, as may be noted in the case of Rats 4 and 7, Period 6. Perhaps a more potent factor in causing this fluctuation is the varying amount of filter paper eaten, as found by Mitchell (57) in an experiment in this laboratory in which rats during one period had no access to filter paper and during the other actually consumed some paper.

It is recognized that the calculation of the metabolic nitrogen in the feces by the method described is subject to an error when applied to a variety of rations. Were the content of crude fiber in all rations the same as in the synthetic nitrogen free ration, the assumption that the metabolic nitrogen of the feces varies directly with the amount of feed consumed would be valid, but with rations varying as widely in the percentage of crude fiber as the corn and alfalfa rations, the adoption of such an assumption evidently leads to an error of undetermined magnitude. However, the method of correcting the absorbed nitrogen and the nitrogen balance by the use of the factor for metabolic fecal nitrogen obtained on nitrogen free rations, undoubtedly gives values nearer the truth than if no such corrections were made, since the actual metabolic fecal nitrogen on the experimental rations containing protein was very probably greater per 100 grams of food consumed than the factors used. In computing the amount of metabolic fecal nitrogen shown in the tables that follow, the values for fecal nitrogen per 100 grams feed eaten in Period 1 in the case of each animal are applied to the data for the same animals in later periods.[1]

[1] These values, as well as those for urinary nitrogen, when being used for these computations, were extended to one more decimal place than shown in Table 12

If it is true, as seems probable from the meager data obtained, that the endogenous metabolism of the rat becomes less intense per unit of live weight as the animal approaches maturity, then in conducting investigations of the kind under consideration it would, no doubt, be advisable to introduce a metabolism period using a nitrogen free ration every six or eight weeks. With these data available it would be possible to make linear corrections for any changes in the requirement of the basal metabolism. In the tables showing the utilization of the protein which follow, such corrections have not been attempted with the limited number of data at hand, but should the data secured with Rats 1 and 4 be used for such a purpose, it would necessitate changes in the utilization coefficients given of not more than one to two per cent as a maximum.

PALATABILITY OF RATIONS. The results obtained in the six metabolism periods during which nitrogenous rations were fed are summarized in Table 13.

From an inspection of the figures giving the amounts of feed consumed daily it is evident that all rations containing cottonseed meal were readily consumed by the animals, attesting to the palatability of this feed, even when forming as much as 24 per cent of the ration. When corn was the sole source of protein in the ration the amounts of feed consumed were smaller than with any other ration. Ground corn seems to be less palatable to rats than whole corn for the latter is usually eaten readily. Perhaps another reason why less of the corn ration was consumed than the alfalfa ration, for example, is that the corn ration was much more digestible and had a higher calorific value so that less of it was required to supply the energy requirements. The ration in which cotton-meal and corn were combined was consumed in greater quantities than the corn ration but not so freely as the cottonseed meal ration. Alfalfa hay proved very palatable, as all rations of which it formed a part were readily eaten.

UTILIZATION OF PROTEINS. In the summary of results shown in Table 13 two methods of calculating the utilization of the proteins fed are included for

TABLE 13.- THE UTILIZATION OF THE PROTEINS OF COTTONSEED MEAL, CORN AND ALFALFA HAY

Summary of Results

Rat No.	Period	Ration	Aver. live wt.	Feed consumed daily	Absorbed nitrogen	Absorbed nitrogen retained	Endogenous nitrogen	Absorbed N retained	Utilization of absorbed nitrogen[1]
			gm.	gm.	mg.	mg.	mg.	pct.	pct.
1	2 + 3	cotton-seed meal	98	9.50	124.8	54.5	25.3	30	64
2	"	"	109	8.62	107.1	38.2	30.4	19	64
3	"	"	127	11.99	169.6	91.3	28.0	44	71
Aver.			111	10.04	133.8	61.3	27.9	31	66
1	4 + 5	cottonseed	114	10.83	125.9	52.9	30.6	26	67
2	"	meal + al-	126	11.06	131.7	58.1	35.7	29	71
3	"	falfa hay	159	15.68	185.0	89.5	33.7	34	67
Aver.			133	12.52	147.5	66.8	33.3	29	68
2	6 + 7	cottonseed meal+alfal-	136	9.82	124.3	45.6	38.2	21	63
3	"	fa hay+corn	186	14.01	173.0	72.5	39.4	27	65
Aver.			161	11.92	148.7	59.0	38.8	24	64
4	2 + 3	corn	118	7.75	118.9	34.6	22.8	15	48
5	"	"	108	6.98	105.9	24.7	27.1	8	49
6	"	"	115	6.92	104.5	34.1	20.3	16	52
Aver.			114	7.22	109.8	31.1	23.4	13	49
4	4 + 5	cottonseed	129	9.81	141.4	59.5	24.9	30	60
5	"	meal + corn	118	8.30	127.8	43.6	29.2	21	59
6	"	"	124	8.11	120.2	48.9	22.3	28	60
Aver.			124	8.74	129.8	50.7	25.5	26	60
5	6 + 7	cottonseed	139	10.90	136.9	49.3	34.9	18	62
6	"	meal+alfal-	141	11.20	146.8	64.8	25.1	28	61
Aver.		fa hay+corn	140	11.05	141.9	57.1	30.0	23	61
7	2 + 3	alfalfa	104	9.42	99.4	37.2	21.4	16	60
8	"	hay	103	8.69	92.9	33.7	20.0	11	58
9	"	"	121	12.55	128.1	61.2	29.4	30	70
Aver.			109	10.22	106.8	44.0	23.6	19	62
7	4 + 5	alfalfa	115	12.64	149.1	56.8	23.3	20	54
8	"	hay +	118	14.08	175.8	78.9	23.3	28	59
9	"	corn	134	12.51	166.6	70.6	32.7	29	62
Aver.			122	13.08	163.8	68.8	26.4	26	58
8	6 + 7	alfalfa	142	11.81	152.6	65.0	27.0	26	61
9	"	hay+corn+	154	13.06	184.0	76.1	38.0	29	62
Aver.		cottonseed meal	148	12.83	168.3	70.6	33.0	27	61

[1] For both maintenance and growth.

comparison, altho for reasons discussed above, the second method, namely, the utilization of the absorbed nitrogen for both maintenance and growth is employed in the discussion here.

The utilization of the nitrogen absorbed from a cottonseed meal ration containing 10 per cent of crude protein was found to be 66 per cent, using the average results of six metabolism periods with three rats. The utilization of the proteins of alfalfa hay was found to be only slightly less than that of cottonseed meal, namely, 62 per cent.

When these two feeds were combined in such proportion that each furnished about an equal amount of digestible protein to the ration, very interesting results were secured, indicating a slight supplementary effect of the proteins from these two sources. This effect was not pronounced, the utilization percentage being 2 per cent above that of cottonseed meal alone and 6 per cent above that of alfalfa hay alone. It is noted that during the periods when the cottonseed meal-alfalfa hay ration was fed, greater quantities of feed were consumed and larger amounts of nitrogen were absorbed than with either the cottonseed meal or alfalfa hay rations alone, which may in some unknown way have operated in effecting a more efficient utilization of the nitrogen, altho the same conditions hold true in the case of both groups of rats which received either the corn or the alfalfa ration, during two periods and were then changed to rations containing proteins from both sources.

It was found that the proteins of corn were utilized the least efficiently of those of the three feedingstuffs compared. When corn was combined with cottonseed meal or with alfalfa hay the resulting utilization coefficients tended toward a mean of the utilization coefficients secured with these feedingstuffs when fed alone, but were nearer that of the feed other than corn. For example, the utilization coefficients found for the corn and cottonseed meal rations were 49 per cent and 66 per cent, respectively, the mean of these two being 57.5 per cent,

but the utilization coefficient found for the cottonseed meal-corn ration was 60 per cent. Possibly this represents a slight supplementary relationship.

The results obtained with the ration in which cottonseed meal, corn and alfalfa hay were combined were remarkably uniform, Of the twelve values obtained with rats receiving this ration during two metabolism periods each, the lowest value was 57 per cent and the highest 67 per cent, the average of all being 63 per cent. The combination of the proteins from three different sources failed to indicate any farther supplementary effect of the proteins.

The high nutritive value of the proteins of cottonseed meal manifested by these experiments is in substantial accord with the conclusions of Richardson and Green (18), Osborne and Mendel (20,21,22,23) and McCollum and Simmonds (24). They do not seem to be in harmony with the findings of Hart and Humphrey (26) who studied the utilization of the proteins of cottonseed meal for milk production, but since growth and milk production are dissimilar functions an absolute comparison of the results of the two experiments is not valid.

CORRELATION OF CHEMICAL COMPOSITION WITH NUTRITIVE VALUE. In seeking for an explanation of the differences in the nutritive value of the proteins of these feedingstuffs based upon differences in their chemical makeup, it is evident first of all that their nutritive values do not vary so widely as the analytical data at hand would indicate. For example, the difference found between the utilization of the proteins of cottonseed meal and alfalfa hay was but 4 per cent, while from an examination of the data in Table 5 it is apparent that cottonseed meal contains more than twice as much arginine nitrogen and nearly twice as much histidine nitrogen as alfalfa hay, while the latter contains more than three times as much nonprotein nitrogen as the former.

Several theories may be advanced in explanation of this apparent inconsistency. In the first place, alfalfa hay is shown to have a lower digestibility than either cottonseed meal or corn. There is no evidence to preclude the pos-

sibility that the character of the nitrogen absorbed from alfalfa hay differs qualitatively from that remaining in the undigested residues. Judging from the ease with which tyrosine is split off from proteins in tryptic digestion in vitro, it is possible that the absorbed nitrogen contains a greater proportion of amino acids essential to the body than the unabsorbed.portion. Further, the stereochemical arrangement of the amino acids in the protein molecule may affect the extent to which the digestive enzymes are able to cause hydrolysis of the different proteins. A second consideration is the possible interchangeability of the various forms of nitrogen in nutrition, as already pointed out in the case of arginine and histidine. To what extent the nonprotein nitrogen of alfalfa hay is utilized in maintenance is problematical, but since there is no reason to doubt that the degradation products of crude protein are able to serve in this capacity, it is possible that a large part of the absorbed nonprotein nitrogen fulfills some of the requirements of the animal body.

It is reasonable to assign the higher content of the basic amino acids of cottonseed meal as the reason for its superiority over the proteins of alfalfa and corn. In the case of the last mentioned feedingstuff, there is the additional factor of a comparatively low lysine content to be considered, altho from the studies of Osborne and Mendel concerning the lysine requirements for growth, as mentioned above, a lysine content of 2.2 per cent of the protein appears to be ample for normal growth.

In the absence of further information respecting the character of the monoamino acid and nonprotein nitrogen content of these feedingstuffs, a detailed picture of which the Van Slyke analysis does not include, correlations between the chemical composition and nutritive value of the proteins of feedingstuffs can proceed little beyohd the realm of the functions and relationships of the basic amino acids.

COMPARISON OF FEED CONSUMPTION WITH THAT OF FARM ANIMALS. It was noted dur-

ing the course of this investigation that the rats consumed an enormous amounts of feed in proportion to their live weights. In some few cases the amount of air dry feed eaten daily was equivalent to as much as 10 or 11 per cent of the live weight. It seemed of interest to compare the feed consumption of albino rats with that of farm animals. Such a comparison is made in Table 14. The tabulations of horses and cattle include in each case two entries of the same group of animals at different ages and weights. It is known that the horses and cattle were restricted in the amount of concentrates consumed but were offered roughage to practically the limit of their appetites. Hence a comparison of the feed consumption of these animals with that of the first group of rats, which were the ones concerned in this investigation, is warranted, but the data are indicative only. Data for the amount of feed consumed by the swine, sheep and second group of rats is not at hand.

It is evident from the data presented that the rat is a voracious eater, even when receiving rations comparable to those of farm animals. A rough approximation places the relative amounts of feed eaten by rats as about three times that of various breeds and classes of farm animals, if swine be excepted. The fact is also brought out, as has previously been noted by others, that the young animal consumes much more feed in proportion to live weight than when older and heavier.

COTTONSEED MEAL PROBABLY NOT TOXIC TO ALBINO RATS. None of the rations containing cottonseed meal seemed to exert any harmful influence upon the rats consuming it. Three of the animals received continuously for 7 weeks rations containing from 7.7 per cent to 28.9 per cent cottonseed meal with no evidence of toxic symptoms but remained in excellent nutritive condition. This observation is in agreement with those of Richardson and Green (18) and Osborne and Mendel (23), as noted in the Introduction.

SUMMARY OF THE DISCUSSION OF THE NUTRITIVE VALUE OF THE PROTEINS. Evidence

TABLE 14.- COMPARISON OF FEED CONSUMPTION BY ALBINO RATS WITH THAT OF FARM ANIMALS

Species or breed and class of animal	Length of feeding period	Feeds in ration	No. of animals fed	Aver. age	Aver. live weight	Feed eaten daily per 100 lbs. live weight
	days			days	lbs.	lbs.
Percheron fillies[1]	28	corn + oats + alfalfa hay	10	227	854	2.2
" "	28	"	10	683	1484	1.8
Hereford steers[2]	35	corn + linseed meal + clover hay	4	---	978	2.5
" "	28	"	4	---	1466	1.5
Jersey heifers[3]	30	corn + bran + linseed oil meal + alfalfa hay	4	365	472	2.9
" "	90	"	4	730	839	1,8
Holstein heifers[3]	30	"	4	365	656	2.4
" "	90	"	4	730	1112	1.8
Swine[4]	--	- - - - - - - - - -	174	---	38	6.0
"	--	- - - - - - - - - -	495	---	128	3.8
"	--	- - - - - - - - - -	105	---	320	2.4
Sheep[5]	--	- - - - - - - - - -	---	---	45	2.1
"	--	- - - - - - - - - -	---	---	127	1.1.
Albino rats	42	cottonseed meal + corn + alfalfa hay + synthetic mixture	9	---	129[6]	8.4[7]
" "[8]	21 or more	18 pct. protein	17	---	175-200[6]	4.6[7]

1 From Bul. 192, Ill. Agr. Exp. Sta.
2 From Bul. 197, " " " " Data concerning "Full-feed lot."
3 From Nebr. Agr. Exp. Sta. Unpublished manuscript. Data concerning "Heavy fed groups."
4 From Henry and Morrison, Feeds and Feeding, 15th Ed. p. 569
5 Calculated from data of Weiske as quoted by Armsby, The Nutrition of Farm Animals, p. 432.
6 Grams
7 Grams per 100 grams live weight
8 From Osborne and Mendel. Protein Minima for Maintenance. J. Biol. Chem., 1915, xxii, 241.

is presented to show that metabolism experiments with the rat as a subject can be carried out with a high degree of adcuracy.

Different methods of expressing the nutritive value of the proteins of feedingstuffs are discussed. The plan of employing the results secured in a preliminary and final metabolism period during which the animal receives a nitrogen free ration, for the calculation of the percentage of the absorbed nitrogen utilized, is favored. In comparing the nutritive value of the combined proteins of the feedingstuffs cottonseed meal, alfalfa hay and corn, it was found that when one of these feeds furnished the sole source of protein in rations containing 10 per cent of crude protein, the utilization of the proteins for growth of albino rats was, in the order in which the feedingstuffs are named, 66 per cent, 62 per cent and 49 per cent,respectively.

When rations containing these feedingstuffs, combined in various ways, but with each feed furnishing an equal amount of digestible protein, were fed, there was evident no clear cut supplementary effect of the proteins of one feed upon another, except in the case of the combination cottonseed meal and alfalfa hay, which showed a slight effect.

No symptoms of toxicity were noted as a result of feeding rations containing cottonseed meal over a period of seven weeks.

When suitable rations are provided, the albino rat consumed an enormous amount of feed in proportion to its live weight.

VI. SUMMARY AND CONCLUSIONS

1. A more complete and detailed, and a more accurate determination of the amino acid content of the proteins of cottonseed meal has been obtainthan has previously been secured.

2. The increase in accuracy of the results is due, first, to the removal of the nonprotein nitrogen before proceeding with the hydrolysis of the protdins; and secondly, to the reduction of the substances formed as a result of the hydrolysis of the proteins.

3. The sum of the basic amino acids was found to be about 0.5 per cent higher than values previously found for cottonseed meal.

4. Of the total nitrogen originally present in the sample, 98.75 per cent was accounted for by summation of the fractions obtained at different stages in the method of analysis, a proportion greater than any previously reported.

5. The method of extraction employed was found to result in the removal of 99.5 per cent of the total nitrogen of the feedingstuff.

6. Metabolism work with rats can be carried out with a high degree of accuracy.

7. In comparing the nutritive value of the combined proteins of the feedingstuffs cottonseed meal, alfalfa hay and corn, it was found that when one of these feeds furnished the sole source of protein in rations containing 10 per cent of crude protein, the utilization of the protein for the growth of albino rats was, in the order in which the feedingstuffs are named, 66 per cent, 62 per cent and 49 per cent, respectively, computed according to the method of Thomas.

8. When rations containing these feedingstuffs, combined in various ways, were fed, there was evident no clear cut supplementary effect of the proteins of one feed upon another, except in the case of the combination cottonseed meal and alfalfa hay, which showed a slight effect.

9. No symptoms of toxicity were noted as a result of feeding rations containing cottonseed meal over a period of 7 weeks.

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VII. BIBLIOGRAPHY

(1) Ritthausen, H. Krystallinische Eiweisskorper aus verschiedenen Oelsamen. Jour. f. prak. Chem. (Neue Folge), 1881, xxiii, 481.

(2) Osborne, T. B. and Voorhees, C. G. The Proteids of Cotton Seed. Jour. Amer. Chem. Soc. 1894, xvi, 778-785; Annual Rpt. Conn. Agr. Expt. Sta., 1893, xvii, 211.

(3) Osborne, T. B. and Harris, I. F. Nitrogen in Protein Bodies. Jour. Amer. Chem. Soc., 1903, xxv, 323-353.

(4) Hausmann, W. Ueber die Vertheilung des Stickstoffs im Eiweissmolekül. Zeit. physiol, Chem., 1899, xxvii, 95-108; Zeit. physiol. Chem., 1900, xxix, 136-145.

(5) Osborne, T. B. The Vegetable proteins. 1912. p. 59.

(6) Abderhalden, E. and Rostoski, O. Die Monoaminosäuren aus Baumwollsamen und dessen Verhalten gegen Magensaft. Zeit. physiol. Chem., 1905, xliv, 265.

(7) Fischer, E. Ueber die Hydrolyze des Caseines durch Salzsäure. Zeit. physiol. Chem., 1901, xxiii, 151-176.

(8) Van Slyke, D. D. The Analysis of proteins by Determination of the Chemical Groups Characteristic of the Different Amino Acids. J. Biol. Chem., 1911, x, 15-55. The Quantitative Determination of Aliphatic Amino Groups. J. Biol. Chem., 1912, xii, 275-284. Improvements in the Method for Analysis of Proteins by Determination of the Chemical Groups Characteristic of the Different Amino Acids. J. Biol. Chem., 1915, xxii, 281-5.

(9) Grindley, H. S., Joseph, W. E., and Slater, M. E. The Quantitative Determination of the Amino Acids of Feeding-stuffs by the Van Slyke Method. J. Amer. Chem. Soc., 1915, xxxvii, 1778-81; Sc., 1915, xlii, 70.

(10) Nollau, E. H. The Amino Acid Content of Certain Commercial Feeding Stuffs and other Sources of protein. J. Biol. Chem., 1915, xxi, 611-14.

(11) Grindley, H. S. and Slater. M. E. The Quantitative Determination of the Amino Acids of Feeding Stuffs by the Van Slyke Method. J. Amer. Chem. Soc., 1915, xxxvii, 2762-9; Proc. Soc. An. Prod., 1917, p. 133.

(12) Benedict, F. G. and Osborne, T. B. The Heat of Combustion of Vegetable Proteins, J. Biol. Chem., 1907, iii, 119-133.

(13) Fraps, G. S. Cottonseed Meal as Human Food. Bul. 128, Texas Agr. Exp. Sta.

(14) Henry, W. A. and Morrison, F. B. Feeds and Feeding. 15th Ed. Appendix Table II.

(15) Mendel, L. B. and Fine. M. S. Studies in Nutrition. V. The Utilization of the Proteins of Cotton Seed. J. Biol. Chem., 1911, xi, 1.

(16) Rather, J. B. Digestion Experiments on Men with Cotton Seed Meal. Bul. 163, 1913, Texas Agr. Exp. Sta. Utilization of the Proteins of Cotton Seed by Man. J. Amer. Chem. Soc., 1914, xxxvi, 584-6.

(17) Pomaski, A. Globulin Obtained from the Seeds of Gossypium hirsutum. Soobshch. Biuro Chastn. Rast. (Petrograd). 1915, ii, No. 2, 3-12; Exp. Sta. Rec., xxxvi, 805.

(18) Richardson, A. E. and Green, H. S. Nutrition Investigations on Cotton Seed Meal. J. Biol. Chem., 1916, xxv, 307-18; J. Biol. Chem., 1917, xxx, 243-258; J. Biol. Chem., 1917, xxxi, 379-388.

(19) Mendel, L. B. Nutrition and Growth. J. Amer. Med. Assoc., 1915, lxiv, 1539.

(20) Osborne, T. B. and Mendel, L. B. Beobachtungen uber Wachstum bei Futterungsversuchen mit isolierten Nahrungssubstanzen. Zeit. fur physiol. Chem., 1912, lxxx, 307-370.

(21) Osborne, T. B. and Mendel, L. B. The Effect of the Amino Acid Content of the Diet on the Growth of Chickens. J. Biol. Chem., 1916, xxvi, 293-300.

(22) Osborne, T. B. and Mendel, L. B. The Relative Value of Certain Proteins and Protein Concentrates as Supplements to Corn Gluten. J. Biol. Chem., 1917, xxix, 69-92.

(23) Osborne, T. B. and Mendel, L. B. The Use of Cotton Seed as Food. J. Biol. Chem., 1917, xxix, 289-317; Proc. Soc. Exp. Biol. and Med., 1915-16, xiii, 147.

(24) McCollum, E. V. and Simmonds, N. A Biological Analysis of pellagra Producing Diets. III. The Values of Some Seed Proteins for Maintenance. J. Biol. Chem., 1917, xxxii, 347-368.

(25) Hart, E. B. and Humphrey, G. C. The Relation of the Quality of Proteins to Milk Production. III. J. Biol. Chem., 1917, xxxi, 445-460.

(26) Hart, E. B. and Humphrey, G. C. The Relation of the Quality of Proteins to Milk Production. IV. J. Biol. Chem., 1918, xxiv 367-383.

(27) Wells, C. A. and Ewing, P. V. Bul. 119, 1916, Ga. Agr. Exp. Sta.

(28) Editorial Exp. Sta. Rec. 1910, xxii, 501-4.

(29) Dinwiddie, R.R. Pig Feeding Experiments with Cottonseed Meal. Bul. 76, 1902, Ark. Agr. Exp. Sta.

(30) Withers, W. A. and Brewster, J. F. Studies on Cottonseed Meal Toxicity. II. Iron as an Antidote. J. Biol. Chem., 1913, xv, 161-166.

(31) Withers, W. A. and Carruth, F. E. Gossypol, the Toxic Substance in Cottonseed Meal. J. Agr. Res., 1915, v, 261; Gossypol, a Toxic Substance in Cottonseed. A preliminary note. Science, 1915, xli, 324; Gossypol, the Toxic Substance in Cottonseed. J. Agr. Res., 1918, xii, 83; Comparative Toxicity of Cottonseed Products. J. Agr. Res., 1918, xiv, 425; Iron as an Antidote to Cottonseed Meal Injury. J. Biol. Chem., 1917, xxxii, 245-258.

(32) Grindley, H. S. and Eckstein, H. C. Reduction of the Quantity of Humin Nitrogen Formed in the Hydrolysis of the Nitrogenous Constituents of Feedingstuffs. J. Biol. Chem., 1919, xxxvii, 373-6. The Free Amid Nitrogen and the Free Amino Acid Nitrogen of Feedingstuffs. Sc., 1915, xlii, 70.

(33) Hamilton, T. S., Grindley, H. S. and Nevens, W. B. The Amino Acid Contents of Feedingstuffs. Unpublished manuscript.

(34) Winterstein, E. Ueber eine Methode sur Abscheidung der organischen Basen aus den Phosphorwolframsaurenniederschlagen und uber das Verhalten des Cystins gegen Phosphorwolframsaure. Zeit. physiol. Chem., 1901, xxxiv, 163-6.

(35) Van Slyke, D. D., Vinograd-Villchur, M. and Losee, J. B. The Abderhalden Reaction. J. Biol. Chem., 1915, xxiii, 377,406.

(36) Hill, R. L. Note on the Use of Colloidal Iron in the Determination of Lactose in Milk. J. Biol. Chem., 1915, xx, 175-7.

(37) Wolff, C. G. L. The Estimation of Non-protein Nitrogen and urea Nitrogen in Blood. J. Physiol., 1915, xlix, 89.

(38) Grindley, H. S. and Eckstein, H. C. The Nonprotein Constituents of Feedingstuffs. J. Amer. Chem. Soc., 1916, xxxviii, 1425-31.

(39) Neidig, R. E. and Snyder, R. S. The Application of the Van Slyke Method to Hydrolyzed Protein Extracts of Silage Crops. J. Amer. Chem. Soc., 1921, xliii, 951.

(40) Gortner, R. A. and Blish, M. J. On the Origin of the Humin Formed by the Acid Hydrolysis of proteins. J. Amer. Chem. Soc., 1915, xxxvii, 1630-6. The Origin of the Humin Formed by the Acid Hydrolysis of Proteins. II. J. Biol. Chem., 1916, xxvi, 177-204.

(41) Gortner, R. A. and Holm, G. E. On the Origin of the Humin Formed by the Acid Hydrolysis of Proteins. J. Amer. Chem. Soc., 1917, xxxix, 2477. The Effect of the Prolonged Acid Hydrolysis upon the Nitrogen Distribution of Fibrin with Especial Reference to the Ammonia Fraction. Ibid, 1917, xxxix, 2736.

(42) Hart, E. B. and Sure, B. The Influence of Carbohydrates on the Accuracy of the Van Slyke Method in the Hydrolysis of Casein. J. Biol. Chem., 1916, xxxiii, 241-249.

(43) Osborne, T. B., Van Slyke, D. D., Leavenworth, C. S. and Vinograd, M. Some products of Hydrolysis of Gliadin, Lactalbumin and the protein

of the Rice Kernel, J. Biol. Chem., 1915, xxii, 259-280.

(44) Lusk, G. L. The Science of Nutrition. 3d Ed. 1919, p. 77.

(45) Menaul, P. A Note on the Modification of the Van Slyke Method of Protein Analysis. J. Biol. Chem., 1921, xlvi, 351.

(46) Ackroyd, H. and Hopkins, F. G. Feeding Experiments with Deficiencies in the Amino Acid Supply; Arginine and Histidine as Possible Precursors of Purines. Biochem. Jour., 1916, x, 551.

(47) Myers, V. C. and Fine, M. S. The Metabolism of Creatine and Creatinine. J. Biol. Chem., 1915, xxi, 389.

(48) Osborne, T. B. and Mendel, L. B. The Comparative Nutritive Value of Certain Proteins in Growth, and the Problem of the Protein Minimum. J. Biol. Chem., 1915, xx, 373.

(49) Geiling, E. M. K. The Nutritive Value of the Diamino Acids Occurring in Proteins for the Maintenance of Adult Mice. J. Biol. Chem., 1917, xxxi, 173.

(50) Mathews, A. P. Physiological Chemistry. 1st Ed. p. 813.

(51) Osborne, T. B. and Mendel, L. B. Amino Acids in Nutrition and Growth. J. Biol. Chem., 1914, xvii, 325.

(52) Osborne, T. B. and Mendel, L. B. The Comparative Nutritive Value of Certain Proteins in Growth, and the problem of the Protein Minimum. J. Biol. Chem., 1915, xx, 351.

(53) Osborne, T. B. and Mendel, L. B. The Amino-Acid Minimum for Maintenance and Growth, as Exemplified by Further Experiments with Lysine and Tryptophane. J. Biol. Chem., 1916, xxv, 1.

(54) Osborne, T. B. and Mendel, L. B. The Amino-Acid Content of the Diet on the Growth of Chickens. J. Biol. Chem., 1916, xxvi, 293.

(55) Withers, W. A. and Fraps, G. S. The Composition of Cottonseed Meal. Bul. 179, 1901, N. Car. Agr. Exp. Sta.

(56) Hart, E. B. and Bentley, W. H. The Character of the Water-Soluble Nitrogen of Some Common Feedingstuffs. J. Biol. Chem., 1915, xxii, 477.

(57) Mitchell, H. H. The Nutritive Value of the Protein Mixtures of Foodstuffs at Different Levels of Intake. Unpublished manuscript.

(58) Osborne, T. B. and Mendel, L. B. The Nutritive Factors in Animal Tissues. J. Biol. Chem., 1917, xxxii, 309-323.

(59) Osborne, T. B. and Wakeman, A. J. Extraction and Concentration of the Water Soluble Vitamine from Brewers' Yeast. J. Biol. Chem., 1919, xl, 383-394.

(60) Waters, H. J. The Capacity of Animals to Grow under Adverse Conditions. proc. Soc. prom. Agr. Sci., 1908, xxix, 3.

(61) Arom. Growth and Nourishment. Biochem. Zeit., 1910, xxx, 207.

(62) Folin. O. Laws Governing the Chemical Composition of Urine. Amer. J. Physiol., 1905, xiii, 66-115; A Theory of Protein Metabolism. Amer. J. Physiol., 1905, xiii, 117-138.

(63) Landergren, E. Untersuchung uber die Eiweissumsetzung des Menschen. Skan. Arch. fur Physiologie, 1903, xiv, 112-175.

(64) Cathcart, E. P. Biochem. Zeit., 1907, vi, 109.

(65) Thomas, Karl. Concerning the Biological Value of the Nitrogenous Substances of Different Foodstuffs. Arch. Anat. Physiol., Physiol. Abt., 1919, p. 219-302.

(66) Rubner, M. Theorie der Ernahrung nach Vollendung des Wachstums. Arch. f. Hygiene, 1908, lxvi, 1-80.

(67) Michaud, L. Beitrag. zur. Kenntnis des physiologichen Eiweissminimum. Zeit. physiol. Chem., 1909, lix, 405-491.

(68) Cathcart, E. P. The Physiology of Protein Metabolism. 1912, p. 68.

(69) Sherman, H. C. Protein Requirement of Maintenance in Man and the Nutritive Efficiency of Bread Protein. J. Biol. Chem., 1920, xli, 97.

(70) Lewis, H. B., Dunn, M. S., and Doisy, E. A. Studies in Uric Acid Metabolism. J. Biol. Chem., 1918, xxxvi, 9.

(71) Mitchell, H. H. The Influence of Protein Feeding on the Concentration of Amino Acids and their Nitrogenous Metabolites in the Tissues. J. Biol. Chem., 1918, xxxvi, 501.

(72) Osborne, T. B. and Mendel, L. B. Amino Acids in Nutrition and Growth. J. Biol. Chem., 1914, xvii, 325.

BIOGRAPHY

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PUBLICATIONS

1. Raising Dairy Calves. Ext. Bul. 51, Nebr. Agr. Exp. Sta., Aug., 1918.

2. Some Factors Affecting the Cost of Milk Production. Ext. Bul. 55, Nebr. Agr. Exp. Sta., June, 1919.

3. The Arrangement of Rectangular Dairy Barns. Cir. 199, Ill. Agr. Exp. Sta. June, 1917. (Jointly with R. S. Hulce).

4. Feed and Care of the Dairy Calf. Cir. 202, Ill. Agr. Exp. Sta., Aug., 1917. (Jointly with R. S. Hulce).

5. Care and Management of the Dairy Herd. Cir. 204, Ill. Agr. Exp. Sta., June, 1919. (Jointly with R. S. Hulce).

6. Purebred Sires Effect Herd Improvement. Cir. 8, Nebr. Agr. Exp. Sta., July, 1919. (Jointly with M. N. Lawritson and J. W. Hendrickson).

7. Dairy Barn and Milk House Arrangement. Cir. 6, Nebr. Agr. Exp. Sta.,

Oct., 1919. (Jointly with J. H. Frandsen.)

8. Breed and Size of Cows as Factors Affecting the Economy of Milk Production. Jour. Dairy Sci. Vol. II, No. 2, Mar., 1919.

9. The Preparation of a Dairy Exhibit. Jour. Dairy Sci., Vol. II, No. 5, Sept., 1919.

10. The Self Feeder for Dairy Calves. Jour. Dairy Sci., Vol. II, No. 6, Nov., 1919.

PUBLICATIONS IN PREPARATION

1. The Amino Acid Contents of the Proteins of Feeding Stuffs. (To be published 1921. Jointly with T. S. Hamilton and H. S. Grindley)

2. The Growth Requirements of Dairy Heifers. (To be published as a bulletin of the Nebr. Agr. Exp. Sta., 1921. Jointly with J. W. Hendrickson, E. G. Woodward and J. H. Frandsen).

3. The Amino Acid Content and Nutritive Value of the Proteins of Cottonseed Meal. (Thesis to be submitted in partial fulfillment of the requirements for the degree of Ph. D. in Animal Husbandry in the Graduate School of the University of Illinois, 1921.)

VIII. APPENDIX

In the analytical work included in the tables below, each cubic centimeter of the standard acid used for nitrogen determinations was equivalent to 0.0014 gram of nitrogen.

Each sample of cottonseed meal taken for analysis weighed 15 grams and contained 1.0194 grams of total nitrogen.

TABLE 1.- TOTAL NITROGEN EXTRACTED BY COLD ABSOLUTE ETHER

Sample No.	Acid neutralized	Nitrogen extracted	Extracted nitrogen as percentage of	
			Total N	Feedingstuff
	cc.	grams	pct.	pct.
C1A	0.15	0.000210	0.021	0.0014
C2A	0.65	0.000910	0.089	0.0061
C3A	1.47	0.002058	0.202	0.0137
C4A	0.80	0.001120	0.109	0.0075
C5A	0.59	0.000826	0.081	0.0055
C6A	0.94	0.001316	0.129	0.0088
C7A	0.59	0.000826	0.081	0.0055
C8A	0.34	0.000476	0.047	0.0032

TABLE 2.- TOTAL NITROGEN EXTRACTED BY COLD ABSOLUTE ALCOHOL

Sample No.	Acid neutralized	Nitrogen extracted	Nitrogen extracted as percentage of	
			Total N	Feedingstuff
	cc.	grams	pct.	pct.
C1B	4.15	0.005810	0.570	0.0387
C2B	4.50	0.006300	0.618	0.0420
C3B	4.75	0.006650	0.652	0.0443
C4B	4.47	0.006258	0.614	0.0417
C5B	3.69	0.005166	0.506	0.0345
C6B	3.56	0.004984	0.489	0.0332
C7B	3.06	0.004284	0.420	0.0285
C8B	3.69	0.005166	0.506	0.0344

TABLE 3.- TOTAL NITROGEN IN THE ONE PER CENT TRICHLORACETIC ACID EXTRACTS AFTER PRECIPITATION WITH COLLOIDAL IRON

Sample No.	Acid neutralized	Volume factor	Nitrogen in filtrate	Nitrogen in filtrate as percentage of	
				Total nitrogen	Feedingstuff
	cc.		grams	pct.	pct.
C1Gf1	3.40	12	0.05712	5.603	0.3808
C1Gf2	3.40	"	0.05712	5.603	0.3808
C1Gf3	3.55	"	0.05964	5.850	0.3976
C2Gf1	3.35	"	0.05628	5.520	0.3752
C2Gf2	3.45	"	0.05796	5.685	0.3864
C2Gf3	3.40	"	0.05712	5.603	0.3808
C3Gf1	3.65	"	0.06132	6.015	0.4088
C3Gf2	3.55	"	0.05964	5.850	0.3976
C3Gf3	3.55	"	0.05964	5.850	0.3976
C4Gf1	3.42	"	0.05746	5.636	0.3831
C4Gf2	3.35	"	0.05628	5.520	0.3752
C4Gf3	3.30	"	0.05544	5.438	0.3696
C5Gf1	4.21	10	0.05894	5.781	0.3930
C5Gf2	4.20	"	0.05880	5.768	0.3920
C5Gf3	3.05	"	0.04270	4.188	0.2846
C6Gf1	4.09	"	0.05726	5.617	0.3817
C6Gf2	4.19	"	0.05866	5.754	0.3911
C6Gf3	4.22	"	0.05908	5.795	0.3940
C7Gf1	4.31	"	0.06034	5.919	0.4023
C7Gf2	4.35	"	0.06090	5.974	0.4060
C7Gf3	4.66	"	0.06524	6.399	0.4350
C8Gf1	4.45	"	0.06230	6.111	0.4153
C8Gf2	5.48	"	0.07672	7.525	0.5115
C8Gf3	5.39	"	0.07546	7.402	0.5031

TABLE 4.- TOTAL NITROGEN IN THE ONE PER CENT TRICHLORACETIC ACID EXTRACTS AFTER SECOND PRECIPITATION WITH COLLOIDAL IRON

Sample No.	Acid neutralized	Volume factor	Nitrogen in filtrate	Nitrogen in filtrate as percentage of	
				Total nitrogen	Feedingstuff
	cc.		grams	pct.	pct.
C1G1a	2.15	16	0.04816	4.724	0.3211
C1G2a	2.25	"	0.05040	4.944	0.3360
C1G3a	2.35	"	0.05264	5.163	0.3509
C2G1a	2.10	"	0.04704	4.614	0.3136
C2G2a	2.25	"	0.05040	4.944	0.3360
C2G3a	2.30	"	0.05152	5.053	0.3435
C3G1a	2.30	"	0.05152	5.053	0.3435
C3G2a	2.75	"	0.06160	6.042	0.4107
C3G3a	2.30	"	0.05152	5.053	0.3435
C4G1a	Lost	--	-------	-----	------
C4G2a	"	--	-------	-----	------
C4G3a	"	--	-------	-----	------
	"				

TABLE 5.- TOTAL NITROGEN IN INSOLUBLE RESIDUES AFTER TREATMENT WITH STRONG SODIUM HYDROXIDE SOLUTION

Sample No.	Acid neutralized	Nitrogen in residues	Nitrogen in insoluble residues as percentage of	
			Total nitrogen	Feedingstuff
	cc.	grams	pct.	pct.
C1FaR	1.60	0.002240	0.220	0.0150
C2FaR	1.90	0.002660	0.260	0.0177
C3FaR	2.20	0.003080	0.302	0.0205
C4FaR	1.70	0.002380	0.233	0.0158
C5FaR	Lost	--------	-----	------
C6FaR	4.99	0.006986	0.685	0.0465
C7FaR	5.24	0.007336	0.719	0.0489
C8FaR	4.29	0.006006	0.589	0.0400

TABLE 6.- INSOLUBLE HUMIN NITROGEN

Sample No.	Acid neutralized	Insoluble humin nitrogen	Insoluble humin nitrogen as percentage of	
			Total Nitrogen	Feedingstuff
	cc.	grams	pct.	pct.
C1La	18.95	0.026530	2.609	0.1769
C2La	19.00	0.026600	2.609	0.1773
C3La	18.15	0.025410	2.492	0.1694
C4La	19.10	0.026740	2.623	0.1783
C5La	21.71	0.030394	2.981	0.2026
C6La	21.34	0.029876	2.930	0.1991
C7La	20.12	0.028168	2.763	0.1878
C8La	20.19	0.028266	2.772	0.1884

TABLE 7.- ACID AMIDE NITROGEN

Sample No.	Acid neutralized	Acid amide nitrogen	Acid amide nitrogen as percentage of	
			Total nitrogen	Feedingstuff
	cc.	grams	pct.	pct.
C1Lb	68.85	0.09639	9.455	0.6426
C2Lb	70.55	0.09877	9.689	0.6585
C3Lb	72.30	0.10122	9.929	0.6748
C4Lb	64.75	0.09065	9.892	0.6043
C5Lb	67.35	0.09429	9.249	0.6286
C6Lb	67.85	0.09499	9.318	0.6333
C7Lb	65.55	0.09177	9.002	0.6118
C8Lb	71.10	0.09954	9.764	0.6635

TABLE 8.- SOLUBLE HUMIN NITROGEN

Sample No.	Acid neutralized	Soluble humin nitrogen	Soluble humin nitrogen as percentage of	
			Total nitrogen	Feedingstuff
	cc.	grams	pct.	pct.
C1Lc	25.21	0.035294	3.462	0.2352
C2Lc	37.26	0.052164	5.117	0.3478
C3Lc	39.75	0.055650	5.459	0.3710
C4Lc	32.60	0.045640	4.477	0.3043
C5Lc	17.59	0.024626	2.415	0.1641
C6Lc	19.30	0.027020	2.650	0.1801
C7Lc	17.00	0.023800	2.334	0.1586
C8Lc	20.00	0.028000	2.746	0.1866

TABLE 9.- PHOSPHOTUNGSTIC ACID HUMIN NITROGEN

Sample No.	Acid neutralized	Volume factor	Nitrogen in entire sample	Humin nitrogen as percentage of	
				Total nitrogen	Feedingstuff
	cc.		grams	pct.	pct.
C1M1PT	7.00	2.5	0.024500	2.403	0.1633
C1M2PT	6.25	"	0.021875	2.145	0.1458
C2M1PT	5.95	"	0.020825	2.042	0.1388
C2M2PT	3.15	"	0.011025	1.081	0.0735
C3M1PT	5.44	"	0.019040	1.867	0.1269
C3M2PT	4.22	"	0.014770	1.448	0.0985
C4M1PT	8.77	"	0.030695	3.011	0.2046
C4M2PT	8.96	"	0.031360	3.076	0.2091
C5M1PT	4.48	2	0.012544	1.230	0.0836
C5M2PT	4.20	"	0.011760	1.153	0.0784
C6M1PT	3.72	"	0.010416	1.021	0.0694
C6M2PT	3.70	"	0.010360	1.016	0.0691
C7M1PT	2.98	"	0.008344	0.818	0.0556
C7M2PT	2.64	"	0.007392	0.725	0.0493
C8M1PT	5.04	"	0.014112	1.384	0.0941
C8M2PT	4.89	"	0.013692	1.343	0.0913

TABLE 10.- NITROGEN SOLUBLE IN AMYL ALCOHOL-ETHER MIXTURE

Sample No.	Acid neutralized	Volume factor	Nitrogen soluble in amyl alcohol-ether in entire sample.	Amyl-alcohol-ether soluble nitrogen as percentage of	
				Total nitrogen	Feedingstuff
	cc.		grams	pct.	pct.
C1M1A-E	2.64	2.5	0.009240	0.906	0.0616
C1M2A-E	3.04	"	0.010640	1.043	0.0709
C2M1A-E	2.14	"	0.007490	0.734	0.0499
C2M2A-E	3.14	"	0.010990	1.078	0.0733
C3M1A-E	1.79	"	0.006265	0.614	0.0418
C3M2A-E	2.80	"	0.009800	0.961	0.0653
C4M1A-E	2.45	"	0.008575	0.841	0.0572
C4M2A-E	4.16	"	0.014560	1.428	0.0971
C5M1A-E	2.35	2	0.006580	0.645	0.0439
C5M2A-E	2.68	"	0.007504	0.736	0.0500
C6M1A-E	2.34	"	0.006552	0.642	0.0437
C6M2A-E	3.34	"	0.003952	0.917	0.0623
C7M1A-E	3.62	"	0.010136	0.994	0.0676
C7M2A-E	3.85	"	0.010780	1.057	0.0719
C8M1A-E	3.82	"	0.010696	1.049	0.0713
C8M1A-E	3.96	"	0.011088	1.087	0.0739

TABLE 11.- NITROGEN IN RESIDUE FROM THE SOLUTION OF THE BASES

Sample No.	Acid neutralized	Volume factor	Nitrogen in residues from entire sample	Nitrogen in residue as percentage of	
				Total nitrogen	Feedingstuff
	cc.		grams	pct.	pct.
C1M1BR	0.60	2.5	0.002100	0.206	0.0140
C1M2BR	0.60	"	0.002100	0.206	0.0140
C2M1BR	0.30	"	0.001050	0.103	0.0070
C2M2BR	0.95	"	0.003325	0.326	0.0222
C3M1BR	0.70	"	0.002450	0.240	0.0163
C3M2BR	0.58	"	0.002030	0.200	0.0136
C4M1BR	0.71	"	0.002485	0.243	0.0165
C4M2BR	0.72	"	0.002520	0.247	0.0168
C5M1BR	0.93	2	0.002604	0.255	0.0173
C5M2BR	2.19	"	0.006132	0.601	0.0409
C6M1BR	1.62	"	0.004536	0.445	0.0302
C6M2BR	1.15	"	0.003220	0.315	0.0215
C7M1BR	0.25	"	0.000700	0.068	0.0047
C7M2BR	0.45	"	0.001260	0.124	0.0084
C8M1BR	0.55	"	0.001540	0.151	0.0103
C8M2BR	0.42	"	0.001176	0.115	0.0078

TABLE 12.- NITROGEN IN RESIDUE FILTERED FROM SOLUTION OF FILTRATE FROM BASES

Sample No.	Acid neutralized	Volume factor	Nitrogen in residue of entire sample	Nitrogen in residue as percentage of	
				Total nitrogen	Feedingstuff
	cc.		grams	pct.	pct.
C1M1FR	0.05	2.5	0.000175	0.017	0.0012
C1M2FR	0.16	"	0.000560	0.055	0.0037
C2M1FR	0.08	"	0.000280	0.027	0.0019
C2M2FR	0.15	"	0.000525	0.051	0.0035
C3M1FR	0.23	"	0.000805	0.079	0.0054
C3M2FR	0.39	"	0.001365	0.133	0.0091
C4M1FR	Lost	"	--------	-----	------
C4M2FR	0.38	"	0.001330	0.130	0.0089
C5M1FR	0.79	2	0.002212	0.217	0.0147
C5M2FR	0.30	"	0.000840	0.082	0.0056
C6M1FR	0.35	"	0.000980	0.096	0.0065
C6M2FR	0.35	"	0.000980	0.096	0.0065
C7M1FR	0.20	"	0.000560	0.054	0.0037
C7M2FR	0.28	"	0.000784	0.076	0.0052
C8M1FR	0.20	"	0.000560	0.054	0.0037
C8M2FR	0.30	"	0.000840	0.082	0.0056

TABLE 13.- ARGININE NITROGEN[1]

Sample No.	a	b	c	d	e	f	g	Arginine nitrogen as percentage of	
	Acid neutralized	Volume after concentration	Volume taken for pptn.	Volume sol. of bases	Volume taken for det'n	Uncorrected arginine nitrogen in sol. of bases	Corrected arginine N in entire sample	Total N	Feed
	cc.	cc.	cc.	cc.	cc.	grams	grams	pct.	pct.
C1M1Ba	12.95	250	100	50	25	0.07252	0.18930	18.569	1.2620
C1M2Ba	13.10	"	"	"	"	0.07736	0.19140	18.775	1.2760
C2M1Ba	13.25	"	"	"	"	0.07420	0.19350	18.981	1.2900
C2M2Ba	13.35	"	"	"	"	0.07476	0.19490	19.119	1.2993
C3M1Ba	12.75	"	"	"	"	0.07140	0.18650	18.295	1.2433
C3M2Ba	13.00	"	"	"	"	0.07280	0.19000	18.638	1.2666
C4M1Ba	13.38	"	"	"	"	0.07493	0.19532	19.160	1.3021
C4M2Ba	12.27	"	"	"	"	0.06871	0.17978	17.635	1.1985
C5M1Ba	16.45	200	"	"	"	0.09212	0.19064	18.701	1.2709
C5M2Ba	14.30	"	"	"	"	0.08008	0.16656	16.339	1.1104
C6M1Ba	17.25	"	"	"	"	0.09660	0.19960	19.580	1.3306
C6M2Ba	17.00	"	"	"	"	0.09520	0.19680	19.305	1.3120
C7M1Ba	15.75	"	"	"	"	0.08820	0.18280	17.932	1.2186
C7M2Ba	15.85	"	"	"	"	0.08876	0.18392	18.041	1.2261
C8M1Ba	17.45	"	"	"	"	0.09772	0.20184	19.799	1.3456
C8M2Ba	18.00	"	"	"	"	0.10080	0.20800	20.404	1.3866

[1] Method of calculation

1. $a \times 2 \times 0.0014 \times \frac{d}{e} = f$

2. $(f + 0.0032) \times \frac{b}{c} = g$

TABLE 14.- AMINO NITROGEN OF THE BASES[1]

Sample No.	a	b	c	d	e	f	g	h	i	j	Amino N of bases as percentage of	
	Vol. of N gas	Temp. degr. C	Bar. press	Blank	Volume after concentration	Volume taken for ppt'n.	Volume sol. of bases	Volume taken for det'n.	Uncorrected amino N in sol. of bases	Corrected amino N in entire sample	Total N	Feed
	cc.		mm.	cc.	cc.	cc.	cc.	cc.	grams	grams	pct.	pct.
C1M1Ba1	7.90	29.0	747.0	0.34	250	100	50	5	0.0403137	0.1137842	11.161	0.7582
C1M1Ba2	8.30	25.0	752.7	0.44	"	"	"	"	0.0431926	0.1209816	11.868	0.8065
C1M2Ba1	8.51	29.0	747.0	0.34	"	"	"	"	0.0435665	0.1219163	11.959	0.8128
C1M2Ba2	8.20	23.0	752.7	0.44	"	"	"	"	0.0430699	0.1206748	11.837	0.8045
C2M1Ba	7.75	27.0	747.0	0.34	"	"	"	"	0.0399584	0.1128960	11.074	0.7526
C2M2Ba	7.40	27.0	747.0	0.34	"	"	"	"	0.0380711	0.1081776	10.611	0.7212
C3M1Ba	8.15	23.0	745.2	0.38	"	"	"	"	0.0426884	0.1197209	11.744	0.7981
C3M2Ba	8.30	25.5	745.2	0.38	"	"	"	"	0.0429580	0.1203950	11.810	0.8026
C4M1Ba	8.85	23.5	745.2	0.38	"	"	"	"	0.0464283	0.1290700	12.661	0.8605
C4M2Ba	8.80	24.0	745.2	0.38	"	"	"	"	0.0460489	0.1281220	12.568	0.8541
C5M1Ba	9.45	23.0	740.3	0.42	200	"	"	4	0.0615987	0.1335970	13.105	0.8906
C5M2Ba1	8.80	22.5	740.3	0.42	"	"	"	"	0.0573218	0.1250430	12.266	0.8336
C5M2Ba2	9.05	23.0	740.3	0.42	"	"	"	"	0.0588700	0.1281400	12.570	0.8543
C6M1Ba	9.20	22.0	740.3	0.42	"	"	"	"	0.0602225	0.1308450	12.835	0.8723
C6M2Ba	9.35	22.0	740.3	0.42	"	"	"	"	0.0612514	0.1329020	13.037	0.8860
C7M1Ba	8.65	22.5	737.0	0.51	"	"	"	"	0.0554283	0.1212560	11.894	0.8084
C7M2Ba	8.70	22.5	737.0	0.51	"	"	"	"	0.0557687	0.1219370	11.961	0.8129
C8M1Ba	9.85	22.5	737.0	0.51	"	"	"	"	0.0635995	0.1375990	13.498	0.9173
C8M2Ba	9.40	22.5	742.4	0.51	"	"	"	"	0.0609854	0.1323700	12.985	0.8825

[1] Method of calculation

1. $\left[(a - d) \times \text{the weight of 1 cc. of nitrogen at the temperature given in (b) and pressure in (e)} \div 1000\right] \times \frac{g}{h} = i$

2. $(i + 0.0052) \times \frac{e}{f} = j$

TABLE 15.- TOTAL NITROGEN OF THE BASES[1]

Sample No,	a Acid neutralized	b Volume after concentration	c Volume taken for ppt'n.	d Volume sol.of bases	e Volume taken for det'n.	f Uncorrected total N in sol. of bases	g Corrected total N in entire sample	Total N of bases as percentage of	
								Total N	Feed
	cc.	cc.	cc.	cc.	cc.	grams	grams	pct.	pct.
C1M1Bc1	7.87	250	100	50	5	0.11018	0.30070	29.497	2.0046
C1M2Bc1	7.79	"	"	"	"	0.10906	0.29790	29.223	1.9860
C2M1Bc1	7.74	"	"	"	"	0.10836	0.29615	29.051	1.9743
C2M2Bc1	7.91	"	"	"	"	0.11074	0.30210	29.635	2.0140
C3M1Bc1	8.24	"	"	"	"	0.11536	0.31365	30.768	2.0910
C3M2Bc1	8.17	"	"	"	"	0.11438	0.31120	30.527	2.0746
C4M1Bc1	8.40	"	"	"	"	0.11760	0.31925	31.317	2.1283
C4M2Bc1	8.35	"	"	"	"	0.11690	0.31750	31.145	2.1166
C5M1Bc1	10.85	200	"	"	"	0.15190	0.32404	31.783	2.1603
C5M2Bc1	10.73	"	"	"	"	0.15022	0.32064	31.453	2.1376
C6M1Bc1	10.89	"	"	"	"	0.15246	0.32512	31.893	2.1675
C6M2Bc1	10.79	"	"	"	"	0.15106	0.32232	31.618	2.1488
C7M1Bc1	10.84	"	"	"	"	0.15176	0.32372	31.755	2.1581
C7M2Bc1	10.76	"	"	"	"	0.15064	0.32148	31.536	2.1432
C8M1Bc1	10.97	"	"	"	"	0.15358	0.32736	32.113	2.1824
C8M2Bc1	11.34	"	"	"	"	0.15876	0.33772	33.129	2.2515

[1] Method of calculation

1. $a \times 0.0014 \times \frac{d}{e} = f$

2. $(f + 0.0101) \times \frac{b}{c} = g$

TABLE 16.- CYSTINE NITROGEN[1]

Sample No.	a Weight of $BaSO_4$	b Weight of blank on reagents	c Volume after concentration	d Volume taken for pptn.	e Volume sol. of bases	f Volume taken for detn.	g Uncorrected cystine N in sol. of bases	h Corrected cystine N in entire sample	Cystine nitrogen as per centage of	
									Total N	Feed
	grams	cc.	cc.	cc.	cc.	cc.	grams	grams	pct.	pct.
C1M1+2B	0.0076	0.0032	250	100	50	10	0.00132020	0.00980049	0.961	0.0653
C2M1B	0.0057	0.0032	"	"	"	"	0.00075011	0.00837527	0.821	0.0558
C2M2B	0.0079	0.0032	"	"	"	"	0.00141021	0.01002550	0.983	0.0668
C3M1B	0.0062	0.0008	"	"	"	"	0.00162024	0.01055060	1.035	0.0703
C3M2B	0.0071	0.0008	"	"	"	"	0.00189028	0.01122570	1.101	0.0748
C4M1B	0.0096	0.0008	"	"	"	"	0.00264039	0.01310100	1.285	0.0873
C4M2B	0.0052	0.0008	"	"	"	"	0.00132020	0.00980050	0.961	0.0653
C5M1B	0.0113	0.0021	200	"	"	"	0.00276041	0.01072080	1.051	0.0715
C5M2B	[illegible]									
C6M1B	0.0098	0.0021	"	"	"	"	0.00231034	0.00982070	0.963	0.0655
C6M2B	0.0093	0.0021	"	"	"	"	0.00216032	0.00952064	0.933	0.0635
C7M1B	0.0042	0.0011	"	"	"	"	0.00093014	0.00706028	0.692	0.0471
C7M2B	0.0047	0.0011	"	"	"	"	0.00108016	0.00736032	0.722	0.0491
C8M1B	0.0062	0.0011	"	"	"	"	0.00153023	0.00826046	0.810	0.0551
C8M2B	0.0052	0.0011	"	"	"	"	0.00123018	0.00766036	0.751	0.0511

[1] Method of calculation

1. $(a - b) \times 0.060009 \times \frac{e}{f} = g$

2. $(g + 0.0026) \times \frac{c}{d} = h$

TABLE 17.- TOTAL NITROGEN IN THE FILTRATE FROM THE BASES[1]

Sample No.	a	b	c	d	e	f	g	Total N in filtrate from bases as percentage of	
	Acid neutralized	Volume after concentration	Volume taken for pptn.	Vol. sol. of bases	Volume taken for det'n.	Uncorrected N in filtrate from bases	Corrected N in entire sample	Total N	Feed
	cc.	cc.	cc.	cc.	cc.	grams	grams	pct.	pct.
C1M1Fa1	43.64	250	100	150	50	0.183288	0.432970	42.473	2.8865
C1M1Fa2	43.84	"	"	"	"	0.184128	0.435070	42.679	2.9005
C1M2Fa1	45.42	"	"	"	"	0.190764	0.451660	44.306	3.0111
C1M2Fa2	44.69	"	"	"	"	0.187698	0.443995	43.554	2.9600
C2M1Fa1	44.69	"	"	"	"	0.187698	0.443995	43.554	2.9600
C2M1Fa2	44.69	"	"	"	"	0.187698	0.443995	43.554	2.9600
C2M2Fa1	45.54	"	"	"	"	0.191268	0.452920	44.430	3.0195
C2M2Fa2	45.46	"	"	"	"	0.190932	0.452080	44.347	3.0139
C3M1Fa1	41.57	"	"	"	"	0.174594	0.411235	40.340	2.7415
C3M1Fa2	43.83	"	"	"	"	0.184086	0.434965	42.668	2.8998
C3M2Fa1	41.24	"	"	"	"	0.173208	0.407770	40.001	2.7185
C4M1Fa1	Lost					-------	-------	------	------
C4M2Fa1	40.31	"	"	"	"	0.169302	0.398005	39.043	2.6534
C4M2Fa2	42.15	"	"	"	"	0.177030	0.417325	40.938	2.7822
C5M1Fa1	56.27	200	"	"	"	0.236334	0.452468	44.385	3.0165
C5M1Fa2	56.41	"	"	"	"	0.236922	0.453664	44.501	3.0243
C5M2Fa1	56.69	"	"	"	"	0.238098	0.455996	44.731	3.0400
C5M2Fa2	56.94	"	"	"	"	0.239148	0.458096	44.937	3.0539
C6M1Fa1	56.24	"	"	"	"	0.236208	0.452216	44.361	3.0148
C6M1Fa2	55.76	"	"	"	"	0.234192	0.448184	43.965	2.9878
C6M2Fa1	56.01	"	"	"	"	0.235242	0.450284	44.171	3.0019
C6M2Fa2	56.12	"	"	"	"	0.235704	0.451208	44.262	3.0081
C7M1Fa1	20.04	"	"	200	25	0.224448	0.428696	42.053	2.8579
C7M1Fa2	20.09	"	"	"	"	0.225008	0.429816	42.163	2.8654
C7M1Fa3	19.99	"	"	"	"	0.223888	0.427576	41.943	2.8505
C7M2Fa1	20.29	"	"	"	"	0.227248	0.434296	42.603	2.8953
C7M2Fa2	20.29	"	"	"	"	0.227248	0.434296	42.603	2.8953
C7M2Fa3	20.19	"	"	"	"	0.226128	0.432056	42.383	2.8804
C8M1Fa1	21.94	"	"	"	"	0.245728	0.471256	46.228	3.1417
C8M1Fa2	21.94	"	"	"	"	0.245728	0.471256	46.228	3.1417
C8M1Fa3	21.79	"	"	"	"	0.244048	0.467896	45.899	3.1193
C8M2Fa1	22.72	"	"	"	"	0.254464	0.488728	47.942	3.2582
C8M2Fa2	22.44	"	"	"	"	0.251328	0.482456	47.327	3.2164
C8M2Fa3	22.74	"	"	"	"	0.254688	0.489176	47.986	3.2612

[1] Method of calculation

1. $a \times 0.0014 \times \frac{d}{e} = f$

2. $(f - 0.0101) \times \frac{b}{c} = g$

TABLE 18.- AMINO NITROGEN IN THE FILTRATE FROM THE BASES[1]

Sample No.	a Vol. N gas	b Temp. degr. C	c Bar. press.	d Blank	e Volume after concentration	f Volume taken for pptn.	g Volume sol. of bases	h Volume taken for det'n.	i Uncorrected amino N in filtrate from sol. of bases	j Corrected amino N of entire sample	Amino N in filtrate as per centage of	
											Total N	Feed
	cc.		mm.	cc.	cc.	cc.	cc.	cc.	grams	grams	pct.	pct.
C1M1Fb1	20.60	28.0	746.1	0.35	250	100	150	10	0.1626808	0.3937021	38.620	2.624
C1M1Fb2	20.50	28.0	746.1	0.35	"	"	"	"	0.1618775	0.3916938	38.424	2.611
C1M1Fb3	20.45	24.0	738.7	0.53	"	"	"	"	0.1619571	0.3918926	38.443	2.612
C1M2Fb1	21.90	24.0	738.7	0.53	"	"	"	"	0.1737461	0.4213652	41.334	2.809
C1M2Fb2	21.90	24.0	738.7	0.53	"	"	"	"	0.1737461	0.4213652	41.334	2.809
C1M2Fb3	22.00	23.0	738.7	0.53	"	"	"	"	0.1753643	0.4254106	41.731	2.836
C2M1Fb1	20.80	23.5	738.7	0.53	"	"	"	"	0.1651827	0.3999569	39.234	2.666
C2M1Fb2	20.95	23.5	738.7	0.53	"	"	"	"	0.1664051	0.4030128	39.534	2.686
C2M1Fb3	-----	----	-----	----					---------	---------	------	-----
C2M1Fb4	20.50	22.0	752.7	0.31	"	"	"	"	0.1689978	0.4094946	40.170	2.730
C2M2Fb1	21.40	22.0	738.5	0.30	"	"	"	"	0.1732442	0.4201105	41.211	2.800
C2M2Fb2	21.60	22.5	738.5	0.30	"	"	"	"	0.1744070	0.4230176	41.496	2.820
C2M2Fb3	21.40	24.0	752.7	0.31	"	"	"	"	0.1747912	0.4239782	41.590	2.826
C3M1Fb1	20.30	24.5	748.2	0.23	"	"	"	"	0.1648700	0.3991750	39.157	2.661
C3M1Fb2	20.40	24.5	748.2	0.23	"	"	"	"	0.1656910	0.4012287	39.359	2.675
C3M1Fb3	20.20	24.5	748.2	0.23	"	"	"	"	0.1640480	0.3971210	38.956	2.647
C3M2Fb1	19.90	23.5	749.5	0.23	"	"	"	"	0.1626830	0.3937070	38.621	2.624
C3M2Fb2	19.90	24.5	749.5	0.23	"	"	"	"	0.1618710	0.3916790	38.422	2.611
C3M2Fb3	20.15	26.0	749.5	0.23	"	"	"	"	0.1625840	0.3934610	38.597	2.623
C4M2Fb1	20.20	22.5	749.5	0.20	"	"	"	"	0.1662370	0.4025930	39.493	2.684
C4M2Fb2	20.50	23.0	749.5	0.20	"	"	"	"	0.1682740	0.4076850	39.992	2.718
C4M2Fb3	20.55	23.5	749.5	0.20	"	"	"	"	0.1683070	0.4077680	40.000	2.718
C5M1Fb1	26.20	21.5	750.1	0.29	200	"	"	"	0.2167010	0.4230030	41.495	2.820
C5M1Fb2	26.40	21.5	750.1	0.29	"	"	"	"	0.2183740	0.4263480	41.823	2.842
C5M2Fb1	27.30	21.0	750.1	0.29	"	"	"	"	0.2265090	0.4426180	43.419	2.950
C5M2Fb2	26.10	21.5	750.1	0.29	"	"	"	"	0.2158650	0.4213300	41.331	2.809
C5M2Fb3	26.60	21.5	750.1	0.29	"	"	"	"	0.2200460	0.4296930	42.151	2.864
C5M2Fb4	26.80	23.0	750.1	0.29	"	"	"	"	0.2199300	0.4294600	42.128	2.863
C6M1Fb1	26.20	21.5	750.1	0.29	"	"	"	"	0.3167010	0.4230030	41.495	2.820
C6M1Fb2	26.40	21.5	750.1	0.29	"	"	"	"	0.2183740	0.4263480	41.823	2.842
C6M2Fb1	26.70	22.0	750.1	0.29	"	"	"	"	0.2202890	0.4301780	42.199	2.867
C6M2Fb2	26.70	22.5	750.1	0.29	"	"	"	"	0.2196940	0.4289890	42.082	2.859
C7M1Fb1	19.00	21.0	742.3	0.37	"	"	200	"	0.2059450	0.4014900	39.384	2.676
C7M1Fb2	18.80	20.5	742.3	0.37	"	"	"	"	0.2042870	0.3981740	39.059	2.654
C7M1Fb3	18.80	21.0	742.3	0.37	"	"	"	"	0.2037340	0.3970680	38.951	2.647
C7M2Fb1	18.90	21.0	742.3	0.37	"	"	"	"	0.2048390	0.3992790	39.168	2.662
C7M2Fb2	19.00	21.0	742.3	0.37	"	"	"	"	0.2059450	0.4014900	39.384	2.676
C8M1Fb1	20.60	20.5	742.3	0.37	"	"	"	"	0.2242390	0.4380780	42.974	2.920
C8M1Fb2	20.70	21.0	742.3	0.37	"	"	"	"	0.2247370	0.4390750	43.072	2.927
C8M2Fb1	21.10	21.0	742.3	0.37	"	"	"	"	0.2291590	0.4479190	43.939	2.986
C8M2Fb2	21.10	21.0	742.3	0.37	"	"	"	"	0.2291590	0.4479190	43.939	2.986

[1] Method of calculation

1. $[(a - d) \times$ the weight of 1 cc. of nitrogen at the temperature given in (b) and the pressure in (e) $+ 1000] \times \frac{g}{h} = i$

2. $(i - 0.0052) \times \frac{e}{f} = j$

TABLE 19.- CALCULATION OF HISTIDINE NITROGEN[1]

Sample No.	a	b	c	d	e	f	Histidine N as percentage of	
	Uncorrected nitrogen of the bases in portion taken for precipitation.				Volume factor	Corrected histidine N in entire sample		
	Total N	Amino N	Arginine N	Histidine N			Total N	Feed
	grams	grams	grams	grams		grams	pct.	pct
C1M1	0.11018	0.0417531	0.07252	0.0210550	2.5	0.0621375	6.095	0.4142
C1M2	0.10906	0.0433182	0.07336	0.0160830	"	0.0497070	4.876	0.3314
C2M1	0.10835	0.0399584	0.07420	0.0191274	"	0.0573185	5.622	0.3821
C2M2	0.11074	0.0380711	0.07476	0.0248984	"	0.0717460	7.038	0.4783
C3M1	0.11536	0.0426883	0.07140	0.0286825	"	0.0812062	7.966	0.5414
C3M2	0.11438	0.0429580	0.07280	0.0252330	"	0.0725825	7.120	0.4839
C4M1	0.11760	0.0464283	0.07493	0.0224640	"	0.0656600	6.441	0.4377
C4M2	0.11690	0.0460489	0.06871	0.0289755	"	0.0819387	8.038	0.5462
C5M1	0.15190	0.0615987	0.09212	0.0318169	2	0.0712339	6.987	0.4749
C5M2	0.15022	0.0580959	0.08008	0.0480961	"	0.1037923	10.181	0.6919
C6M1	0.15246	0.0602225	0.09660	0.0296812	"	0.0669625	6.568	0.4464
C6M2	0.15106	0.0612514	0.09520	0.0276129	"	0.0628258	6.163	0.4188
C7M1	0.15175	0.0554283	0.08820	0.0452725	"	0.0981451	9.627	0.6543
C7M2	0.15064	0.0557687	0.08876	0.0424519	"	0.0925039	9.074	0.6167
C8M1	0.15358	0.0635995	0.09772	0.0250357	"	0.0576715	5.657	0.3845
C8M2	0.15876	0.0609854	0.10080	0.0332619	"	0.0741238	7.271	0.4942

[1] Method of calculation

1. $1.5 (a - b) - 1.125 = d$

2. $(d + 0.0038) \times e = f$

TABLE 20.- CALCULATION OF LYSINE NITROGEN[1]

Sample No.	a	b	c	d	e	f	g		
	Uncorrected nitrogen of the bases in portion taken for precipitation.					Volume factor	Corrected lysine nitrogen in entire sample	Lysine N as percentage of	
	Total N	Arginine N	Cystine N	Histidine N	Lysine N			Total N	Feed
	grams	grams	grams	grams	grams		grams	pct.	pct.
C1M1	0.11018	0.07252	0.00132020	0.0210550	0.0152848	2.5	0.0394620	3.871	0.2631
C1M2	0.10906	0.07336	0.00132020	0.0160830	0.0182968	"	0.0469920	4.609	0.3133
C2M1	0.10836	0.07420	0.00075011	0.0191274	0.0142825	"	0.0369563	3.625	0.2464
C2M2	0.11074	0.07476	0.00141021	0.0248984	0.0096714	"	0.0254284	2.494	0.1695
C3M1	0.11536	0.07140	0.00162020	0.0286825	0.0136573	"	0.0353932	3.472	0.2360
C3M2	0.11438	0.07280	0.00189028	0.0252330	0.0144568	"	0.0373920	3.668	0.2493
C4M1	0.11760	0.07493	0.00264039	0.0224640	0.0175676	"	0.0451690	4.430	0.3011
C4M2	0.11690	0.06871	0.00132020	0.0289755	0.0178923	"	0.0459808	4.510	0.3065
C5M1	0.15190	0.09212	0.00276041	0.0318169	0.0252027	"	0.0514054	5.042	0.3427
C5M2	0.15022	0.08008	0.00276041	0.0480961	0.0192835	"	0.0395670	3.881	0.2638
C6M1	0.15246	0.09660	0.00231034	0.0296812	0.0238684	"	0.0487369	4.780	0.3249
C6M2	0.15106	0.09520	0.00216032	0.0276129	0.0260867	"	0.0531735	5.216	0.3545
C7M1	0.15176	0.08820	0.00093014	0.0452725	0.0173573	"	0.0357147	3.503	0.2381
C7M2	0.15064	0.08876	0.00198016	0.0424519	0.0183479	"	0.0376958	3.697	0.2513
C8M1	0.15358	0.09772	0.00153023	0.0250357	0.0292940	"	0.0595891	5.845	0.3972
C8M2	0.15876	0.10080	0.00213018	0.0332619	0.0234679	"	0.0479358	4.702	0.3196

[1] Method of calculation

1. $a - (b + c + d) = e$

2. $(e + 0.0005) \times f = g$

TABLE 21.- NON-AMINO NITROGEN IN THE FILTRATE FROM THE BASES[1]

Sample No.	a	b	c	d	e	Non-amino N as percentage of	
	Uncorrected nitrogen in the filtrate from the bases in portion taken for precipitation.			Volume factor	Corrected non-amino nitrogen in entire sample	Total N	Feed
	Total N	Amino N	Non-amino N				
	grams	grams	grams		grams	pct.	pct.
C1M1	0.183708	0.1621717	0.021536	2.5	0.041591	4.079	0.2773
C1M2	0.189231	0.1742855	0.014946	"	0.025114	2.463	0.1674
C2M1	0.187698	0.1668618	0.020836	"	0.039841	3.908	0.2656
C2M2	0.191100	0.1741475	0.016953	"	0.030131	2.955	0.2009
C3M1	0.197345	0.1648690	0.014476	"	0.023940	2.348	0.1596
C3M2	0.173208	0.1623790	0.010829	"	0.014823	1.454	0.0988
C4M1	0.173168	0.1676060	0.005562	"	0.001655	0.161	0.0110
C4M2	0.173166	0.1676060	0.005560	"	0.001655	0.161	0.0110
C5M1	0.236628	0.2175370	0.019091	"	0.028382	2.784	0.1892
C5M2	0.238623	0.2205870	0.018036	"	0.026272	2.577	0.1751
C6M1	0.235200	0.2175370	0.017663	"	0.025526	2.504	0.1702
C6M2	0.235473	0.2199910	0.015482	"	0.021164	2.076	0.1411
C7M1	0.224448	0.2046550	0.019793	"	0.029786	2.921	0.1986
C7M2	0.226874	0.2053920	0.021482	"	0.033164	3.253	0.2211
C8M1	0.245168	0.2244880	0.020680	"	0.031560	3.095	0.2104
C8M2	0.253493	0.2291590	0.024334	"	0.038868	3.812	0.2591

[1] Method of calculation

1. a - b = c

2. (c - 0.0049) x d = e

TABLE 22.- THE UTILIZATION OF THE PROTEINS OF COTTONSEED MEAL, CORN AND ALFALFA HAY[1]

Rat No.	Period	Ration	Initial weight	Final weight	Daily feed consumed	a Daily intake of N	b Daily urinary N	c Daily fecal N	d Metabolic N in feces	e Endogenous N	f Absorbed N retained	g Utilization of absorbed N[3]	h Absorbed N retained	i Digestibility: Ordinary coefficient	j Digestibility: Corrected coefficient
			gm.	gm.	gm.	mg.	mg.	mg.	mg.	mg.	mg.	pct.	pct.	pct.	pct.
1	2	cottonseed meal	92	103	9.52	166.7	71.8	62.2	24.1	24.4	56.8	63	31	63	77
2	2	"	104	113	8.66	151.6	70.7	62.2	22.1	30.4	40.8	64	21	59	74
3	2	"	121	133	11.51	201.4	74.3	68.3	29.7	26.8	88.5	71	44	66	71
Aver.												66	32	63	74
1	3	"	103	109	9.48	165.8	68.8	68.9	24.0	26.1	52.1	65	29	58	73
2	3	"	113	118	8.57	149.9	67.0	69.2	21.9	30.4	35.6	64	17	54	69
3	3	"	133	144	12.47	218.1	82.2	74.1	32.3	29.2	94.1	70	43	66	81
Aver.												66	30	59	74
1	4	cottonseed	109	113	10.60	189.8	68.6	100.1	26.9	29.7	48.0	67	24	47	62
2	4	meal + alfal-	118	129	11.39	203.9	67.2	96.1	29.1	34.6	69.7	76	38	53	67
3	4	fa hay	145	160	14.91	267.0	91.6	132.4	38.7	32.2	81.7	66	32	50	65
Aver.												70	31	50	65
1	5	"	113	121	11.06	198.0	77.4	90.9	28.0	31.4	57.7	66	28	54	68
2	5	"	129	133	10.73	192.1	80.0	93.0	27.4	36.7	46.5	66	19	52	66
3	5	"	160	173	16.45	294.5	99.3	140.6	42.7	35.1	97.3	67	35	53	67
Aver.												66	27	53	67
1	6	N-free	113	102	6.10	0	21.5	18.7	----	----	----	--	--	--	--
2	6	cottonseed	134	137	10.13	180.5	79.8	78.1	25.8	37.9	48.4	67	22	57	71
3	6	meal + alfal-	182	188	14.47	257.9	102.2	119.1	37.5	39.0	74.1	64	26	54	69
Aver.		fa hay + corn										66	24	56	70
2	7	"	137	138	9.51	169.4	77.7	73.3[2] (65.5)	24.3	38.5	42.7 (50.5)	67 (69)	19	57 (61)	71 (76)
3	7	"	188	189	13.54	241.3	98.8	106.8	35.1	39.8	70.8	65	27	56	70
Aver.												62	23	57	71
4	2	corn	117	119	7.44	123.4	80.3	28.5	18.6	22.7	32.2	49	15	77	92
5	2	"	108	108	7.66	127.2	81.7	30.1	20.9	27.1	36.3	54	16	76	93
6	2	"	116	115	7.49	124.3	70.4	32.4	20.2	20.4	41.7	55	23	74	90
Aver.												53	18	76	92
4	3	"	119	119	8.05	133.6	88.3	29.5	20.2	22.8	36.0	47	15	78	93
5.	3	"	108	107	6.30	104.6	80.8	28.0	17.2	27.0	13.0	43	--	73	90
6	3	"	115	113	6.35	105.3	70.4	25.6	17.2	20.2	26.5	48	9	76	92
Aver.												46	12	76	92

TABLE 22.- THE UTILIZATION OF THE PROTEINS OF COTTONSEED MEAL, CORN AND ALFALFA HAY[1] (Continued)

Rat No.	Period	Ration	Initial weight	Final weight	Daily feed consumed	a Daily intake of N	b Daily urinary N	c Daily fecal N	d Metabolic N in feces	e Endogenous N	f Absorbed N retained	g Utilization of absorbed N[3]	h Absorbed N retained	i Digestibility: Ordinary coefficient	j Digestibility: Corrected coefficient
			gm.	gm.	gm.	mg.	mg.	mg.	mg.	mg.	mg.	pct.	pct.	pct.	pct.
4	4	corn + cotton-	122	131	9.95	176.8	80.4	61.1	24.9	24.3	60.2	60	31	66	80
5	4	seed meal	110	115	8.56	152.0	80.0	52.4	23.3	28.2	42.9	58	20	66	81
6	4	"	116	128	8.48	150.8	76.2	49.6	22.9	21.6	47.9	56	25	67	82
Aver.												58	25	66	81
4	5	"	131	135	9.67	171.9	83.5	53.9	24.2	25.5	58.7	59	29	69	83
5	5	"	115	125	8.04	142.8	78.5	42.0	21.9	30.1	44.2	61	22	71	86
6	5	"	128	132	7.73	137.4	66.4	42.0	20.9	23.0	49.9	63	30	69	85
Aver.												61	27	70	85
4	6	N-free	135	130	9.02	0	16.3	20.1	----	----	----	--	--	--	--
5	6	cottonseed	134	139	11.03	196.6	88.5	92.2	30.1	34.3	46.1	60	15	53	68
6	6	meal + corn +	133	143	10.68	190.3	77.3	88.0	28.9	24.4	53.9	60	24	54	69
Aver.		alfalfa										60	20	54	69
5	7	"	139	144	10.77	191.9	86.8	82.1	29.4	35.5	52.4	63	21	57	73
6	7	"	143	148	11.72	208.9	86.8	78.2	31.7	25.8	75.6	62	33	63	78
Aver.												63	27	60	76
7	2	alfalfa hay	103	109	9.33	171.0	61.0	91.4	24.8	21.3	43.4	62	23	47	61
8	2	" "	103	105	9.19	165.8	62.5	91.1	27.4	20.0	39.6	58	16	45	62
9	2	" "	116	119	11.42	206.3	65.4	122.5	28.1	28.8	46.5	67	22	41	54
Aver.												62	20	44	59
7	3	" "	109	104	9.50	171.5	63.4	102.4	25.2	21.4	30.9	57	8	40	55
8	3	" "	105	103	8.20	148.0	55.9	88.7	24.4	20.0	27.8	57	6	40	57
9	3	" "	119	126	13.67	246.9	68.4	136.3	33.7	30.0	75.9	73	38	45	58
Aver.												62	17	42	57
7	4	alfalfa hay	107	117	12.86	219.6	95.1	105.3	34.2	22.5	53.4	51	17	52	68
8	4	+ corn	106	125	14.82	253.1	105.8	111.9	44.2	22.2	79.6	55	25	56	73
9	4	"	127	132	12.18	208.0	94.5	79.6	30.0	31.7	63.9	60	26	62	76
Aver.												55	23	57	72
7	5	"	117	122	12.41	211.9	89.4	95.3	33.0	24.0	60.2	57	23	55	71
8	5	"	125	129	13.34	227.8	88.0	101.5	39.8	24.4	78.1	62	30	55	73
9	5	"	132	140	12.84	219.3	97.5	76.1	31.6	33.3	77.3	63	32	65	80
Aver.												61	28	58	75
7	6	N-free	118	105	4.76	0	23.8	17.0	----	----	----	--	--	--	--
8	6	cottonseed meal	134	141	12.06	214.9	93.2	96.0	36.0	26.4	61.7	57	22	55	72

TABLE 22.- THE UTILIZATION OF THE PROTEINS OF COTTONSEED MEAL, CORN AND ALFALFA HAY[1] (Continued)

Rat No.	Period	Ration	Initial weight	Final weight	Daily feed consumed	a Daily intake of N	b Daily urinary N	c Daily fecal N	d Metabolic N in feces	e Endogenous N	f Absorbed N retained	g Utilization of absorbed N[3]	h Absorbed N retained	i Digestibility: Ordinary coefficient	j Digestibility: Corrected coefficient
			gm	gm	gm	mg	mg	mg	mg	mg	mg	pct.	pct.	pct.	pct.
9	6	+ corn + alfalfa	147	157	13.55	241.5	109.2	86.0	33.3	37.2	79.6	62	30	64	78
Aver.												60	26	60	75
8	7	"	141	147	11.55	205.8	82.0	90.1	34.4	27.6	68.3	64	29	56	73
9	7	"	157	160	12.57	224.0	106.6	75.8	31.0	38.8	72.6	62	28	66	80
Aver.												63	29	61	78

[1] Method of calculation

1. $a - b - (c - d) = f$
2. $\frac{e + f}{a - (c - d)} \times 100 = g$
3. $\frac{a - c - b}{a - c} \times 100 = h$
4. $\frac{a - c}{a} \times 100 = i$
5. $\frac{a - (c - d)}{a} \times 100 = j$

[1] A loss occurred during Kjeldahl digestion of feces

[2] Based on amount of fecal nitrogen per gram of food in Period 6

[3] For both maintenance and growth.